DOCTOR OF PHILOSOPHY

Diagnostic Accuracy and Reproducibility of Conventional and Novel Caries Detection Methods as Determined by Histology and Micro-CT; A Study In Vitro

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Diagnostic Accuracy and Reproducibility of Conventional and Novel Caries Detection Methods as Determined by Histology and Micro-CT; A Study *In-Vitro*

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DDS, MDSc

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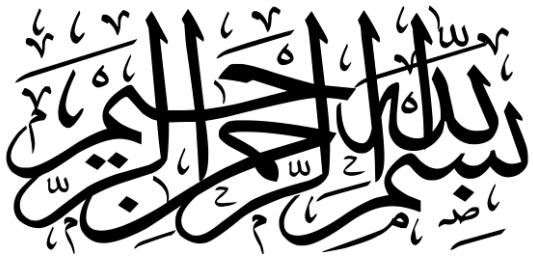

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ

Recite in the name of your Lord
Who created all creatures

خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ

Created man from a clinging substance.

اقْرَأْ وَرَبُّكَ الْأَكْرَمُ

Recite, and your Lord is the most Generous

الَّذِي عَلَّمَ بِالْقَلَمِ

Who taught by the pen

عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ

Taught man that which he knew not

Table of Contents

List of Tables

List of Figures

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Declaration

I, Tamer Saleh ALJamaan, hereby declare that I am the author of this thesis and that all the references cited have been consulted by myself. I was the principal investigator in all studies described in this thesis. This work has not previously been submitted for a higher degree in this or any other university.

Tamer Saleh ALJamaan

Certificate

I hereby certify that Tamer Saleh ALJamaan has fulfilled the conditions of Ordinance 39 of the University of Dundee and is qualified to submit this for Doctor of Philosophy.

Professor David Ricketts

Dr. Andrew Hall

Having a dream is what keeps you alive. Overcoming the challenges make life worth living"

Mary Tyler Moore, 1936

For my wife and my daughters:

Your pride in me was infinite, your trust unequivocal and your love eternal.

You are always in my heart, I love you.

Abstract

Background: Dental caries is considered as one of the most common human diseases. In its early stages, it can be treated preventively where the lesion can be arrested or even remineralised. As such, early caries detection and monitoring is required to determine the severity and activity of the lesion over time and to evaluate the effectiveness of the preventive treatment modalities. Traditionally, caries detection is carried out primarily with a visual examination and bitewing radiographs (BW). Other caries detection devices have been developed to provide objective measurements, for example the DIAGNOdent and more recently the DIAGNOdent Pen, and a newer device, the CarieScan PRO, which relies on Electrical Impedance Spectroscopy.

Over the last three decades, there has been a revolution in the medical CT technology in parallel with the invention of compact machines such as cone beam CT which can be used in the dental practice by producing three dimensional images of the teeth and related structures. Micro-CT is considered a novel (3D) digital technology that shows great promise for detecting subtle changes within structures, and has the potential to be an alternative to routine histological sectioning in laboratory studies for validation of caries detection methods without the need for associated destruction of teeth.

Aims and Objectives:

1. To determine the reproducibility of conventional visual (ICDAS) and BW radiographs and novel caries detection methods (Fiberoptic Transillumination (FOTI), Cone Beam-CT (CBCT), DIAGNOdent Pen (DD) and CarieScan PRO (CS) for the detection of occlusal and proximal caries in permanent teeth.

2. To evaluate the relationship between histological sections and corresponding Micro-CT tomographic slices, for the assessment of the presence and extent of dental caries on both the occlusal and proximal surfaces in permanent premolar and molar teeth.

3. To evaluate the diagnostic accuracy of the caries detection methods as determined by histological and Micro-CT validation.

4. To devise a quantitative method for volumetric analysis of occlusal and proximal lesions from Micro-CT images and to develop a novel method to determine the percentage mineral loss in the lesion.

Materials and Methods: A preliminary inspection was carried out to select 200 teeth with a total of 244 occlusal and 400 proximal investigation sites. Eleven examiners were recruited to take part in this study, who were all qualified and experienced dentists. All examinations were carried out with teeth set up in anatomical arches except for CarieScan PRO where teeth were examined individually. Subsequently, 140 teeth underwent Micro-CT scanning and then all teeth were serially sectioned in a mesio-distal direction. For qualitative analysis of lesions, a precise and reliable locating technique was developed to accurately link occlusal investigation sites to histological section and corresponding Micro-CT image. For quantitative analysis of lesions, a new method was devised for caries lesion area measurement and volumetric analysis in three dimensions using Micro-CT. In addition, a novel method was developed for the measurement of mineral content within the enamel of carious lesions.

Results:

1. The inter- and intra-examiner reproducibility of the DIAGNOdent Pen and ICDAS were superior to the other examination methods for occlusal and proximal caries detection

respectively and showed strong to substantial agreement. However, the CarieScan PRO and the DIAGNOdent Pen were inferior to other examination methods for occlusal and proximal caries detection respectively showing poor agreement between and within examiners.

2. Using two currently accepted histological classification systems (Downer and Ekstrand, Ricketts, Kidd (ERK)), there was a strong relationship and agreement between Micro-CT tomographic images and corresponding histological sections.

3. In relation to diagnostic accuracy, the ICDAS and CBCT were superior compared with other methods for occlusal and proximal caries detection.

4. The diagnostic accuracy of DIAGNOdent Pen and digital BW radiographs were poor at both the D_1 and D_3 diagnostic thresholds for occlusal and proximal caries detection.

5. There were highly significant differences in the area, volume and percentage of mineral loss between enamel and dentine lesions on the occlusal surfaces according to Downer classification system. However, the differences were less significant on the proximal surfaces.

Conclusion: ICDAS has the greatest potential of the detection methods tested for clinicians to use in clinical practice. The use of the DIAGNOdent Pen and CarieScan PRO as an adjunct over and above ICDAS is questioned. CBCT also performed well in relation to diagnostic accuracy, and whilst it should not be used solely for the purpose of caries detection, it is important to report on dental caries if requested for any other clinical reason. For research purposes, Micro-CT would be a valuable method for validation of new caries detection methods based on quantitative measurements rather than qualitative depth analysis of caries lesions.

CHAPTER ONE

Introduction and Literature review

1.1 Introduction

Dental caries is one of the most prevalent worldwide chronic disease of humans and is considered as a global health problem in both industrialised and developing countries. The prevalence is still high among children, adolescents and young adults and in most countries the disease affects nearly 100% of the population, affecting approximately five billion people worldwide(Petersen and Yamamoto, 2005). Literature on dental and oral infections goes back to the ancient Romans, Greeks and Egyptians. However, it was only in the late 19th and 20th centuries that the field of dentistry was revolutionised. The aetiology of dental caries and its progression were proposed and investigated but its details are still being researched today (Simón-Soro and Mira, 2015).

Dental caries is a microbial disease characterised by a localised destruction of enamel induced by the acidic by-product from bacterial fermentation of dietary carbohydrates. The caries process is dynamic due to biofilm-tooth interactions that can occur over time on and below a tooth surface. This process involves a shift in the balance between protective factors (that aid in remineralisation) and destructive factors (that aid in demineralisation) in overall favour of demineralisation of the tooth structure over time. The process can be arrested at any time (Hanada, 2000; Longbottom *et al.*, 2009a; Fontana *et al.*, 2010). However, the caries process begins long before any signs appears clinically on the tooth surface (Zandoná and Zero, 2006).

The current status of the disease in some developed, privileged, populations is characterised by low prevalence and slow progression (Pitts *et al.*, 1998), however, it is dynamic in nature and in its early stages lesions can be arrested or even remineralised leading to a state of so-called caries reversal (Murdoch-Kinch and McLean, 2003). Such

detection of early lesions (i.e. before cavitation) as well as monitoring lesion progress is now an important goal of clinical dentistry (Lussi, 1993; Anusavice, 2005).

1.1.1 Mechanisms Involved in Caries Process:

The aetiology of dental caries is complex and multifactorial, with contributions from nutrition, tooth morphology, fluoride exposure, microbial ecology, salivary flow, oral hygiene, and other factors that remain to be defined (Cummins, 2013; Punitha *et al.*, 2015) The magnitude each of these factors contributes to caries can vary significantly on an individual basis. It has been known for over 100 years that dental caries is caused by bacteria-fermenting foods, producing acids and dissolving tooth mineral (Krause, 1953).

Bacterial Involvement The critical role of microorganisms was confirmed following the Second World War, initially when antibiotics were shown to prevent caries. It was not until 1954 that experiments confirmed dental caries is a microbial disease. Most of these early studies concluded that dental caries is an infectious as well as transmissible disease (Orland, 1959; Littman and Williams, 2005).

The most recent ecological plaque hypothesis by (Marsh, 2008) proposed that dental caries is a naturally occurring phenomenon that takes place in dental biofilm. Because of the natural occurrence of these organisms, as well as others, it is widely accepted that an imbalance in the number of these bacteria with respect to the total bacterial count plays an integral role in the development of dental caries (Loesche, 1986). The prevalence of mutans streptococci and lactobacilli is highly correlated with the incidence of dental caries (Keene *et al.*, 1976). Dental plaque could be more cariogenic locally, where mutans streptococci and lactobacilli are concentrated, especially with the development of highly species-specific monoclonal IgG antibodies against S. mutans. These tests are sensitive

enough to detect a single bacterial cell in saliva (Yee and Sheiham, 2002)which would help in the clinical diagnosis and treatment of caries (Shi *et al.*, 1998).

Sugar Consumption Dietary carbohydrates are necessary for bacteria to produce the acids that initiate the demineralization process (Paes Leme *et al.*, 2006). The most studied part of carbohydrates in relation to caries are sugars, and in particular sucrose, which has been widely accepted as main cause in caries aetiology (Bradshaw and Lynch, 2013). In general, the role of diet to induce caries lesions is based on three factors: the drop in environmental pH, the frequency of ingestion, and the cariogenicity of foods (Featherstone, 2006). The pH at which this demineralization begins is known as the critical pH and ranges between pH 5.0 and 5.5 (Harper and Loesche, 1986).

Oral Cavity Environment Saliva composition and volume are very important in creating a symbiotic relationship between host and microflora species (Mark Steven and Charlie, 2009). Salivary flow helps to neutralize and clear the acids and carbohydrates from dental plaque and has several functions including a specific flushing effect, the maintenance of calcium super-saturation in plaque, the neutralisation of acids, raising the plaque pH and reversing the diffusion of calcium and phosphate from deeper within the tooth, toward the tooth surface (Lenander-Lumikari and Loimaranta, 2000).

Demineralization/Remineralization Enamel exposed to the oral environment experiences constant surface changes and modifications due to the recurring changes in pH generated by diet and plaque accumulation. During acid exposures, calcium and phosphate are precipitated out from the tightly packed hydroxyapatite lattice leading to demineralization of tooth structure. As the acid is buffered and the pH levels begin to rise, Calcium and Phosphate are re-incorporated into the Hydroxyapatite matrix causing the tooth to remineralize (Kidd and Fejerskov, 2004). These phases of demineralization and

remineralization, when in balance, result in no net mineral loss, therefore preserving the structural integrity of the tooth surface. However, such changes in ion content influence the physical and chemical properties of the mineral and, most importantly with respect to enamel, change its solubility. It is this process that is desired when incorporating fluoride into drinking water and toothpaste (Slade *et al.*, 2013).

The process of balance/imbalance between such factors could explain why tooth surfaces which are constantly covered by biofilm do not always develop a carious lesion, because the outcome of complex interactions within the biofilm between different species of microbes may not necessarily result in mineral loss.

Simply, dental caries is a microbial disease, resulting from acids released due to metabolism of dietary carbohydrates of the oral bacteria in the biofilm at tooth surface.

1.1.2 The Prevalence of Dental Caries:

The World Health Organization (WHO) in 1981, set ambitious goals for reducing the prevalence of dental caries in young children for the next 20 years. These goals included 86% of children would not lose any teeth, 50% of 5–6 year old children would be caries free and the world average of decayed, missing or filled permanent teeth (DMFT) among 12 year olds children would be less than 3 (WHO, 1981).

Although dental caries prevalence in industrialized countries has declined over the past several decades, the prevalence is still high (Petersen and Yamamoto, 2005). This is attributed to several of factors, which include the increase in intake of cariogenic foods, limited exposure to fluorides, low socioeconomic status, ethnicity, health in general, age, access to oral health services, and other lifestyle factors (Miura *et al.*, 1997).

With these factors in mind, new goals have been set for 2020, by WHO and World Dental Federation (FDI) who issued "Global Goals for Oral Health 2020" (Hobdell *et al.*, 2003). These goals provide guidance to policy makers to improve the oral health status of their populations. Each country may specify targets according to its current disease prevalence.

The effect of dental caries on overall quality of life and well-being has not been extensively studied in the context of developing countries. In developing countries, the disease is too expensive to manage with conventional invasive treatment procedures. According to WHO, the cost of conventional treatment of dental caries in developing countries would exceed the total health care budget for children, if available (Yee and Sheiham, 2002).

Although caries prevalence has declined among children and adolescents in many countries (World Health Organization 2003), it still remains a problematic issue in the Middle East including Kingdom of Saudi Arabia (KSA). The caries experience among preschool children was reported to be 72% in the United Arab Emirates and Jordan (al Mughery *et al.*, 1991; Janson and Fakhouri, 1993). Another study showed that the mean number of decayed, missing, and filled surfaces (DMFS) of a random sample of 1,096 adult Jordanian patients was 34.9. All subjects had coronal caries experience and 93% had untreated lesions(Hamasha and Safadi, 2008).

Dental caries is more prevalent in the lower socioeconomic groups and among some ethnic groups (Truin *et al.*, 1998). Some studies have shown that for the prediction of caries development, social and demographic factors could be useful in very young children, but for older individuals, clinical parameters are more predictive (Demers *et al.*, 1992).

Due to the increase in preventive treatment modalities such as the emergence of fluoridated drinking water and toothpastes, there has been a dramatic decrease in smooth surface caries resulting in an increased proportion of occlusal caries when evaluated in the context of total caries prevalence (Alwas-Danowska *et al.*, 2002). Early occlusal caries detection has become more difficult due to the absence of cavitation in and underneath fissures as a result of frequent fluoride use (Weerheijm *et al.*, 1997). Because of this fact, today there is a greater need for an effective ways for early occlusal caries detection.

In developed countries, dental caries affect 60-90% of school-aged children and most adults (Petersen and Yamamoto, 2005). Over the years, evidence has suggested a decline in the prevalence of dental caries, particularly among children, adolescents and young adults in developed countries(Szöke and Petersen, 2000). In Lithuania, a series of cross-sectional studies conducted between 1993 and 2001, involving 12- and 15-year-olds, revealed a decrease in the mean DMFT scores of both age groups in both high- and low-fluoride areas. In low-fluoride areas, the DMFT among 12-year-olds declined from 5.8 to 4.5, whereas in high-fluoride areas, the mean DMFT declined from 2.6 to 1.9 (Aleksejuniene *et al.*, 2004). Contrary to the optimistic view that caries is disappearing, stabilization in caries experience has been reported in some countries. In Finland, the mean DMFT among 15-year-olds declined sharply from 12.1 in 1976 to 3.6 in 1990 and then stabilized at 3.0 in 1993 (Vehkalahti *et al.*, 1997).

A Surveys coordinated by the British Association for the Study of Community Dentistry (BASCD) in 1996/97 conducted among 12 years old in the United Kingdom caries was detected caries into dentine (D_3) threshold using a visual method. This survey demonstrated, once again, a wide variation in caries prevalence across the United

Kingdom. Overall, 44% of children had evidence of caries experience at the dentinal level (D_3) of detection. These findings demonstrate the continuing need for more effective preventive strategies and treatment modalities for this important age group (Pitts *et al.*, 1998).

The first national survey of children's dental health in England and Wales was carried out in 1973. The 2013 survey involved England, Wales and Northern Ireland. It aimed to consider all surveys, from 1973 to 2013, so as to summarise trends in the dental health of children in the UK over the last 40 years. The final analysis of the data collected since 1973 showed that caries prevalence had reduced from 72% to 41% in 5-year-olds and from 97% to 46% in 15-year-olds, over the 40- year-period (Murray *et al.*, 2015).

A survey, among 11-12 year-old students, was undertaken in Scotland, Wales and England in 2008/09, using BASCD standard criteria to detect caries at the dentinal threshold, using visual criteria. The trend for reducing caries prevalence and severity continued in this age group in all three countries (Davies *et al.*, 2012).

1.1.3 Clinical Presentation of Caries:

The earliest clinically visible sign of dental caries is the white spot lesion which is evident after two weeks of undisturbed biofilm formation in contact with tooth surface (Holmen *et al.*, 1985b; Holmen *et al.*, 1985a). There will be a slight increase in the porosity of the outer microsurface of enamel to form what is so-called, “subsurface demineralisation” (Kidd and Fejerskov, 2004). After one month of biofilm formation, the classical histological zones can be recognized under the polarized light microscope. These zones are the surface zone, the body of the lesion, the dark zone and the translucent zone (Siverstone, 2009). The earliest white spot lesion can be seen only on clean and dry teeth.

The explanation is related to different refractive indices of enamel, water and air. The refractive index of enamel is 1.62 (Spitzer and Ten Bosch, 1975). When demineralization occurs, the porosities within enamel are filled with water having a refractive index of 1.33. This difference in the refractive index between water and enamel will cause the tissue to lose its translucency due to an increase in the backscattered light. If the enamel was dried, the water will be replaced with air of refractive index 1.0, which is greater than difference between water and enamel and so the lesion is now even more opaque or chalky in appearance (Thylstrup *et al.*, 1994). However, with the advancement of lesions, the porosities within enamel increase and the effect becomes obvious even under wet conditions. The shape of the white-spot lesion in enamel is determined by the direction of enamel prisms and the surface area covered by the biofilm. For instance, on the proximal surface, a kidney-shaped area can be found at the contact-point area or sub-contact-point area above the gingival margin which is conical in shape with the base is towards the surface (Ekstrand *et al.*, 1995). On the occlusal surface the lesions are formed in three dimensions at the deepest part of the fissure and guided by prism direction. The shape of the lesion would be conical and spreads on all sides of the fissure with the base towards the enamel-dentine junction (EDJ) (Ekstrand *et al.*, 1997). Thus, although the fissure may appear healthy, extensive caries may still be present underneath it. Dentine will react due to stimulation, as a result of acid diffusion through the microporous enamel. This reaction is considered as a defence mechanism and leads to deposition of minerals within dentinal tubules to form so-called tubular sclerosis (Stanley *et al.*, 1983). However, clinically, the brownish discolouration of dentine will be evident once the enamel lesion reaches the EDJ (Ismail, 1997). As the lesion progresses, micro-cavitation of enamel can occur with demineralisation of the underlying dentine. Some carious dentinal lesions may

manifest as a chalky-white, brown or greyish/bluish shadow showing through the apparently intact enamel but, when frank cavitation occurs, various degrees of discoloration, ranging from chalky-white, white-yellowish, to brown or dark discolouration, may be seen (Fejerskov, 2004). This phenomenon is partly responsible for the problem of under-detection of occlusal dentine lesions. Previous studies showed, under ideal visual conditions, dentists only detect between 20% and 48% of lesions penetrating in dentine. More lesions can be detected radiographically. These lesions are called "hidden caries" (Kidd *et al.*, 1993; Ketley and Holt, 1993).

Generally, the non-cavitated lesion only progresses to cavitation upon the complete breakdown of overlying enamel. No serious microbial invasion takes place in the dentin as long as the highly organized enamel layer (even though being demineralized) separates the biofilm from the dentine (Bjørndal, 2008).

The presence of fluoride has had a major impact on the prevention of enamel breakdown as a result of its antibacterial properties, its ability to prevent demineralization and its ability to aid in remineralization (Jones *et al.*, 2005). As a result of fluoride use lesions present clinically with histological extension into dentine, despite the appearance of intact enamel due to the presence of fluoride.

It is important to mention that not all opacities and/or discolorations are attributed to dental caries. These presentations are indicative of a lower mineral content within the enamel, however it is possible that these manifestations are attributed to a number of different mechanisms during enamel formation i.e. hypomineralization or extrinsic staining (He *et al.*, 2010).

1.1.4 Management of Dental Caries:

The management of the disease undertaken by most dentists, in the twentieth century ,was the removal of the demineralised tissue, regardless of the level of infection and histological destruction, and placement of a restoration for oral health improvement (Ismail *et al.*, 2001). Owing to the changes in the pattern of lesion progression, the low prevalence of caries and reduced potential risk of initial lesions progressing to cavitated caries lesions (Mejàre *et al.*, 1999), further caries development should to be a reason for finding new strategies for caries prevention. This is in line with Nyvad (2004), who concluded that "the time is now ripe for a move from operative to non-operative care in the management of dental caries with the essential aims of arresting and healing the caries lesions at an early stage". The intention should be primary prevention, i.e. to prevent even non-cavitated proximal initial caries lesions (Pitts, 2004a; Blinkhorn and Davies, 2013).

The rationale behind operative management Operative treatment is performed in order to aid plaque control, maintain pulp vitality (which might be endangered by bacterial toxins), prevent drifting of teeth (due to loss of proximal contacts), and most importantly restoral the oral health status of the individual (McComb, 2005; Ricketts *et al.*, 2013). Cavitated lesions require operative treatment, especially when they are on occlusal and proximal surfaces, due to the difficulty of plaque removal, making oral hygiene control ineffective in these areas.

A Cochrane review by Ricketts, *et al,* (2009), comparing complete or ultraconservative removal of decayed tissue in unfilled teeth, concluded that complete caries removal caused significantly more damage to the pulp and the ultraconservative approach did not adversely affect the longevity of the restorations.

Rationale and Treatment approach to Minimally Invasive Dentistry (Stepwise excavation of dentine caries) Minimally Interventional Dentistry should be used for prevention and early caries detection to help prevent the disease from progressing further and to avoid any unnecessary destructive treatment. If treatment is necessary, then the least invasive option should be used to control the disease process.

The primary aim of minimally-invasive treatment should be to eradicate only the highly infected and irreversibly demineralised enamel and dentine (Banerjee, 2000). For extensive lesions, the primary aim would be to leave just enough pulpal dentine to reduce chance of pulp exposure (Opal *et al.*, 2014). It has been demonstrated that the number of bacteria decreases during stepwise excavation procedures, and results in the lesion arresting clinically when restored with an adhesive material such as glass ionomer cement. A second aim would be a final excavation stage to remove the slowly progressing but still slightly infected discoloured demineralised dentine, before the permanent and definitive restoration is placed.

Traditional preventive treatment includes oral hygiene instructions; pit and fissure sealants; fluoride application (by patient or professionally applied); and dietary assessment and appropriate advice (Longbottom *et al.*, 2009a). New strategies have evolved such as laser treatment to increase enamel resistance to dissolution and the Hall technique, which represents a hybrid combination of secondary and tertiary prevention, have also been proposed (Longbottom *et al.*, 2009b).

1.1.5 Relevance of Caries Diagnosis in Modern Clinical Caries Management:

The diagnosis of caries is an integral part of dentistry used by clinicians in every day practice. Problems of caries detection and diagnosis are now being encountered with the

apparent changes in the presentation and the distribution of the lesions. However, it is important in the first step to differentiate between the stages lead to caries diagnosis before the application of appropriate and available treatment options. There is considerable confusion with the terminology employed in the literature. The modern caries management is composed of several and discrete stages. An essential first step is caries diagnosis (Pitts, 2004b). However, this term is a collection of several parameters which include:

(1) **Lesion detection**, an objective method of determining whether or not disease is present based on subjective criteria or objective measurements. This is essentially a yes/no decision. The result obtained depends upon several factors, including: the true state of the tooth surface with a reference method, also known as a "gold standard". The accuracy of the method, the reliability in clinical use, and the influence of any detection threshold that has been chosen.

(2) **Lesion measurement** to define the stage of the caries process. Caries measurement methods must take into account a thorough understanding of the histopathological morphology and appearance of different sizes and types of lesion according to certain diagnostic thresholds on the basis of the depth of penetration of the caries through the tooth tissues

(3) **Lesion monitoring**: the aim of modern dentistry is to characterise or monitor a lesion, once it has been detected. This is used at a series of examinations when lesions are less advanced than the particular stage judged to require operative intervention. Such monitoring can traditionally be made from serial standardized clinical examinations or serial radiographic examinations (Tranæus *et al.*, 2005). Attempts to monitor small

changes in lesions over time led to a recognition of the need to measure such changes with objective and, it was hoped, more reproducible and accurate methods

(4) **Caries activity assessment** clinicians ideally need to be able to measure the dynamic activity of each individual lesion to differentiate its current behaviour whether active or inactive. Some promising modern criteria for clinical visual assessment include estimates of lesion activity from a single clinical examination is available (Ekstrand *et al.*, 2007; Braga *et al.*, 2010b; Nyvad *et al.*, 1999).

The **caries diagnosis**: is based on all the information obtained from steps 1 through 4 which are later synthesized. It should be emphasized that true diagnosis is not possible without taking into account individual patient factors and caries risk status which are important considerations in these decisions.

Simply, caries diagnosis is a process of: detection of the lesion, followed by an assessment of the severity of the lesion, which again is followed by an assessment of the activity. The patient risk assessments and the overall health of the patient should have a role in making decision of the appropriate diagnosis and treatment options.

Assessment of lesion activity is very important so that preventive techniques can be applied to those individuals in need. For example, Nyvad *et al,* (1999) developed a visual tactile caries diagnostic system to determine caries activity. Nyvad's system focuses on surface characteristics rather than the depth of the lesion. The surface texture is an indicator of actual caries activity. The surface integrity is dependent on the presence of any cavitation's or activity within the enamel.

An active non-cavitated lesion is characterized as a "whitish/yellowish opaque surface with a loss of luster, exhibiting a chalky appearance" (Fejerskov2009). The surface feels

rough when explored. The inactive version of this lesion conversely is shiny and feels smooth when explored. The colour can vary from whitish to dark in colour, however colour is generally considered a non-reliable indicator. An active cavitated lesion appears soft and leathery, while an inactive lesion appears shiny and is hard on probing.

This study will explore all the aspects of caries detection and lesion measurement and the term of caries detection will be used further in the study.

1.2 Caries Detection Methods

Diagnosis of dental caries and deciding when to provide treatment is still considered very subjective and continues to be widely debated. There is a considerable research regarding this subject matter in an attempt to better understand the caries progression process and how it can be prevented. To complicate the matter, dental caries has become increasingly more difficult to diagnose since the widespread use of fluoride. Non-cavitated dentinal lesions are far more prevalent as a result of minimal demineralization on occlusal surfaces making visual detection much more difficult (Sawle and Andlaw, 1988; Weerheijm *et al.*, 1989).

The ability to quantitatively measure the progression of caries would be a useful clinical tool to objectively identify the extent of the disease process. Additionally, a predictable quantitative measure could improve caries research by ensuring uniformity in lesion progression without relying on inter-rater reliability. The following literature review will address conventional i.e. visual and bitewing radiograph (BW) and novel detection methods i.e. DIAGNOdent (laser fluorescence) and CarieScan PRO (electrical impedance).

Detection of dental caries has been viewed as an iceberg (Pitts, 2004b) where the earliest changes i.e. the subclinical demineralisation and remineralisation in enamel are at the hidden base of this iceberg. This dynamic lesion is difficult if not impossible to detect. At the next level lesions can be detected but require additional diagnostic aids. At the tip of the iceberg is the lesion that can be easily detected clinically, for example, a cavity (Figure 1.1). Usually carious lesions are detected by vision which can be aided by a number of traditional and advanced lesion detection aids. In view of the above, this thesis will deal only with noncavitated lesions.

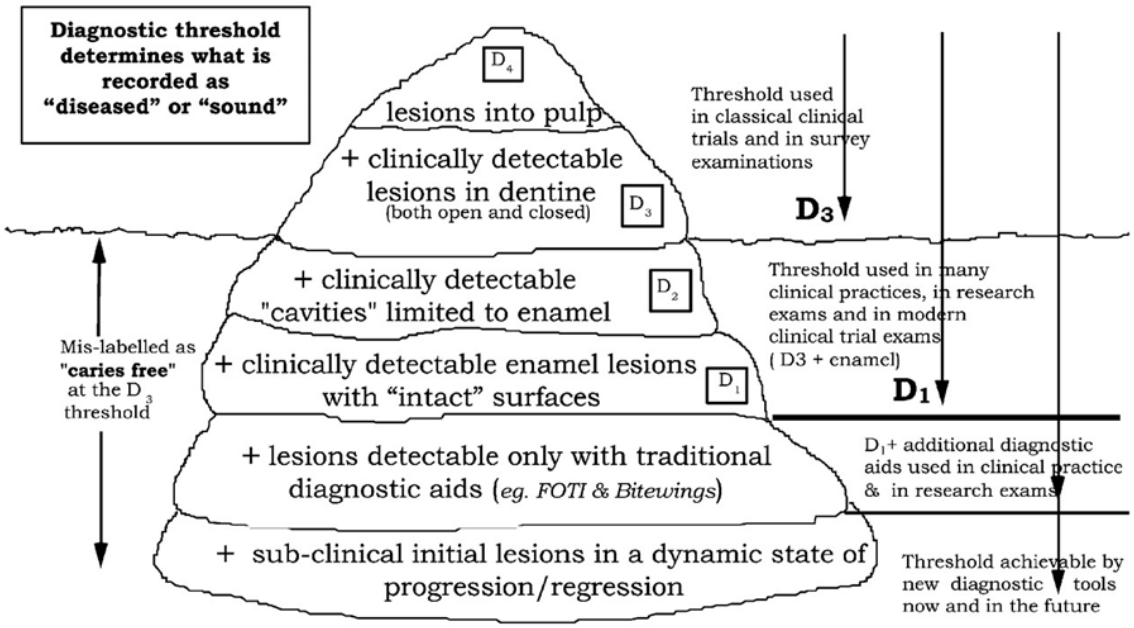


Figure 1.1 The "iceberg of dental caries"-diagnostic thresholds in clinical trials and practice by courtesy of (Pitts, 2004b).

1.2.1 Visual Examination Method:

Visual examination is the most commonly used method for detecting caries lesions, as it is an easy technique that is routinely performed in clinical practice (Kidd *et al.*, 1993; Pitts, 1993). The most commonly used method in clinical practice and epidemiological

surveys, for the detection of carious lesions, is visual and visual/tactile examination which is carried out in a dental chair or portable chair with the aid of normal light, a blunt probe and 3 in 1 syringe. This method depends on the examiner's interpretation (subjectivity) of the findings which normally leads to a wide variation in terms of diagnostic accuracy (Bader *et al.*, 2002). Traditionally, visual inspection has high levels of specificity, but low sensitivity and reproducibility. It is believed the levels of low reproducibility are attributed to the subjective nature of caries detection (Braga *et al.*, 2010a; Ismail, 2004). As a measure to improve on low sensitivity and reproducibility values, several indices have been created. These will be discussed further in this section.

Visual/Tactile detection method A sharp-end explorer has traditionally been used to check for stickiness of pits and fissures for detection of caries. Lussi, (1991) investigated the diagnostic accuracy and treatment decisions on occlusal pits and fissures using visual and visual/tactile methods on 61 posterior teeth which were examined by 34 dentists. It was found that the specificity of dentists using an explorer was slightly higher (87.4%) than the specificity of dentists who did not (82.5%). The sensitivity however, was slightly less (60.5%) with the explorer than without (65%). These findings were not found to be significantly different, however, dentinal caries lesions were frequently missed by this tactile method showing no improvement over the visual method. In (2004), Ismail undertook a comprehensive review of the literature on published studies on visual and visual-tactile methods of caries detection. This review was focused on the content validity of a sample of reported caries detection criteria. This review confirmed the lack of consistency regarding explorer use, and again confirmed that explorer use added little to caries detection while posing a possibility of detriment to enamel surfaces. Additionally, there is adequate literature to support the disadvantages of probing fissures such as

transfer of cariogenic bacteria and/or producing irreversible damage in the demineralised tissue and further cavity formation (Ekstrand *et al.*, 1987; van Dorp *et al.*, 1988). In order to improve accuracy, as well as reproducibility, of the visual/tactile method for detection and assessment of lesion depth from the early stages of the caries process, several subjective visual scoring systems were developed demonstrating appropriate levels of reliability when used by experienced and calibrated examiners (Pitts and Fyffe, 1988; Ismail *et al.*, 1992; Ekstrand *et al.*, 1995). In addition, some of these criteria included lesion activity assessments (Nyvad *et al.*, 1999; Fyffe *et al.*, 2000; Ekstrand *et al.*, 2007). In spite of the limitations of the method, it has been stated that the visual/tactile examination is the only approach to date that would allow a differentiation between non-cavitated and cavitated carious lesions, as well as between active and inactive lesions; these are the pivotal factors on which the best caries treatment options can be determined (Baelum *et al.*, 2012).

Historical perspectives and the need for an integrated system

Scientists have been trying to develop a reliable and reproducible diagnostic system for the detection and diagnosis of dental caries by clinical visual examination. Since 1954, there have been systems which included codes for the diagnosis of non-cavitated caries as well as the cavitated (Dirks, 1961).

In the early 1970s, the World Health Organisation (WHO) started publishing its reports about basic methods for oral health surveys (WHO, 1981). The WHO system is one of the most widely used systems in epidemiological surveys. Their diagnostic threshold for caries diagnosis is the cavitation level. The current WHO (1997) diagnostic criteria are:

- Caries is recorded as present when a lesion in a pit or fissure, or on a smooth tooth surface, has a detectably softened floor, undermined enamel, or softened wall.
- A tooth with a temporary filling should also be included in this category.
- On proximal surfaces, the examiner must be certain that the explorer has entered a lesion.
- When any doubt exists, caries should not be recorded as present.

It has been shown that the detection of caries at the cavitated level results in an underestimation of caries levels in the population studied especially with the exclusion of enamel lesions (Groeneveld, 1985; Manji *et al.*, 1989).

As such, Pitts and Fyffe (1988) conducted a study to test the effect of inclusion (or exclusion) of initial enamel lesions on the results of a clinical examination in a low caries prevalence group. The level of decay reported was almost doubled when the initial caries and enamel caries were included.

Efforts of scientists thus continued in order to provide a more reliable system for caries detection (Neilson and Pitts, 1991; Fyffe *et al.*, 2000; Ekstrand *et al.*, 1998; Nyvad *et al.*, 1999).

A comprehensive review, based on content validity (i.e. does the method describe adequately what is being described about the lesion?) was conducted by Ismail (2004) from a sample papers of visual caries detection criteria, since 1950. He concluded that the content validity demonstrated considerable variation among visual scoring systems in terms of the disease process being measured, inclusion and exclusion criteria, and examination conditions.

Prior to this comprehensive review, a systematic review was undertaken by Bader (2002) to identify the performance of different methods for detecting carious lesions, one of which was the visual examination. His review revealed that visual and visual/tactile methods for caries detection achieved wide variation of sensitivity between 0.1 and 0.95. He also found that the strength of evidence available for estimation of the validity of the visual examination for caries diagnosis is poor.

International Caries Detection and assessment System (ICDAS) In order to develop an integrated clinical detection and assessment system of dental caries, a group of caries researchers, restorative dentists, paediatric dentists and epidemiologists assembled to update the caries detection and assessment criteria and to put together all different definitions. A new system thus was developed in 2002, which was named the International Caries Detection and assessment System (ICDAS), following two development meetings in Dundee, Scotland (April, 2002) and Ann Arbor, Michigan (August, 2002) (Larato).

The development of ICDAS I and ICDAS II criteria was based on the research conducted by Ekstrand *et al*., (1995) and (1997) combined with work by Nyvad and colleagues (1999) and concepts from the Dundee Selectable Threshold Method (DSTM) for caries diagnosis (Fyffe *et al.,* 2000), in addition to other caries detection systems which were described by Ismail (2004) in his systematic review.

ICDAS is a clinical visual scoring system which was developed for use in clinical practice, research, epidemiology and dental education. The main aim for the development of the ICDAS was to provide an international system which allows standardisation of data collection and enables comparability between studies (Ismail *et al.,* 2007).

Based on the workshop for the development of ICDAS criteria in 2002, the decision was taken in 2005 to change to ICDAS II by switching the original code 3 and 4 of ICDAS I This decision was taken in Baltimore, USA in 2005.

The recording of dental caries using the ICDAS system is a two stage process. The code consists of two digits, the first digit is the restorative status of the tooth and the second digit is for the caries severity. ICDAS codes for caries severity are shown in Table 2.2 (Topping and Pitts, 2009)

Ekstrand *et al.*, in 2007, attempted to devise a scoring system for the assessment of caries activity, named Lesion Activity Assessment (LAA), of coronal lesions by combining the results obtained from the visual appearance of the lesion according to ICDAS II classification, the location of the lesion in the plaque stagnation area and the tactile sensation (rough/soft or smooth/hard) using a ball ended periodontal probe. Furthermore, they attempted to validate the findings by using that Clinpro impression material which changes its colour at the pH threshold 5.5. The system showed adequate reproducibility in terms of percent agreement and was good indicator to differentiate between active and inactive lesions. In another study, comparing the LLA with Nyvad scoring system, the LLA seemed to overestimate the activity of the cavitated lesions according to the cumulative score of the clinical parameters especially with the lack of a reference method to assess the accuracy of both systems. However, both systems showed comparable results in terms of reliability and validity for the detection of lesion severity (Braga *et al.*, 2010a). Further experiments are required to determine the feasibility of LAA to be combined with ICDAS scoring system.

ICDAS has been proved to be useful in epidemiological surveys by improving its sensitivity (with the inclusion of non-cavitated lesions) which in turn shortens the

duration of clinical trials. Moreover, it provides comparable results in comparison with WHO system(WHO, 1997). However, longer time and more cost is needed especially in large surveys (Braga *et al.*, 2009b; Kühnisch *et al.*, 2008).

ICDAS preventively oriented the framework for patient caries management into a more comprehensive International Caries Classification and Management System (ICCMS) (Pitts and Ekstrand, 2013). It is designed to accommodate the needs of different users across the ICDAS domains of clinical practice, dental education, research and public health. These options are represented as 2012 ICDAS / ICCMS wardrobe (*www.icdas.org*). The process focuses on the caries severity detection and activity assessment which is followed by a health outcomes protocol based on prevention-focussed clinical care for individual patients, control of initial non-cavitated lesions, and conservative restorative treatment of deep dentinal and cavitated caries lesions. While ICDAS provides feasible methods for classifying severity of caries and the activity status of lesions, ICCMS provides choices to enable dentists to integrate and synthesise tooth and patient information, including caries risk status, in order to improve long-term caries outcomes.

Using ICDAS scoring system is feasible to be used in combination with other detection methods such as Quantitative Light-Induced Fluorescence (QLF) and Bitewing Radiographs (Rodrigues *et al.*, 2008; Zandoná *et al.*, 2010) for the detection of occlusal lesions. This combination has the potential to improve the performance of ICDAS system although it needs to be validated for its reliability.

In addition, ICDAS has shown a reasonable reliability and validity in the detection of carious lesions especially the enamel lesions on the occlusal surfaces of permanent teeth

(Ismail *et al.*, 2007; Ekstrand *et al.*, 2007; Zandona *et al.*, 2009; Jablonski-Momeni *et al.*, 2008).

An example of International recognition to promote and support the ICDAS system worldwide is the Alliance for a Cavity-Free Future (ACFF). This group of worldwide leaders from the dental and public health professions have joined together to create a "global Alliance" (http://www.allianceforacavityfreefuture.org) promoting integrated clinical and public health action in order to stop caries initiation and progression and move towards a cavity-free future by working with countries to build on existing initiatives with a new understanding of caries as a disease continuum and to adopt comprehensive strategies for caries prevention and management on the basis of communities or individuals. The mission of this global social activity is to 'Stop Caries NOW for a Cavity-Free Future'. It promotes population awareness of the full continuum of dental caries and uses ICDAS methodology, particularly in supporting developments in dental education in developing countries.

A recent systematic review by Gimenez *et al*, (2015b)conducted to evaluate several visual scoring systems including ICDAS for caries detection. The authors concluded that widely recognised and validated visual detection methods had good overall accuracy for caries detection for both laboratory and clinical studies on proximal and occlusal surfaces. This systematic review highlighted the validity and accuracy of using ICDAS as a detailed visual scoring system for caries detection in every day dental practice.

1.2.2 Conventional and Digital Radiographic Examination Method:

Before considering the use of radiographs for the detection of carious lesions, we should bear in our mind the risks accompanied with the use of ionizing radiation. The guide

principle should be weighed against risks and all unnecessary exposure to radiation should be limited (Pitts and Kidd, 1992).

Conventional bitewing radiographs A profound studies over the last three decades has confirmed the importance of bitewing radiographs of proximal lesions. The situation with occlusal caries is rather different due to the superimposition of buccal and lingual sound enamel in the fissures make the detection of enamel lesion impossible although some *in vitro* studies claimed the possibility for enamel lesions to be seen in radiographs (Tveit *et al.*, 1994; Neuhaus *et al.*, 2009a). Regarding dentine lesions, clinicians reported that lesions visible in dentine on Bitewing radiographs are larger when treated operatively. This would mean that radiographs underestimate lesions and it would be regarded more than a safety net. There is controversy in the literature regarding the accuracy and benefits of Bitewing radiographs over the visual examination (Ketley and Holt, 1993; Ricketts *et al.*, 1995a; Pitts and Fyffe, 1991). Such studies have shown high numbers of false positives, which would mean an over-estimation of the caries present. However, the specificity of this method was high when compared with visual examination as the latter is based on subjective interpretation of the caries. With the decline of caries prevalence among the populations, the impact of false positive cases are less (Downer, 1989; Wenzel *et al.*, 1993). The under-estimation of radiographs of dentine lesion size would mean that radiographs should not relied upon for monitoring of caries progression. When the practitioners first alter the profession in UK in the early 1980s to give attention for occlusal caries detection, there was little evidence to support the usage of radiographs in combination with clinical examination (Dooland and Smales, 1982).

With the changes in the pattern of caries, radiographs are becoming more important for occlusal caries diagnosis especially at cavitation diagnostic threshold at the first stage

especially in epidemiological surveys (Weerheijm *et al.,* 1992; Creanor *et al.,* 1990). It should be noted that most of these studies were *in vivo* and there is lack of operative or histological gold standard. For this reason the number of false positives is not precise.

A number of *in vitro* studies have attempted to improve radiographic caries detection (Wenzel *et al.,* 1991b; Ricketts *et al.*, 1995a; Wenzel *et al.*, 1991a). Most of these studies have enhanced by the BW by defining the edges of the lesions, using xeroradiographs and over-exposed BW radiographs. These enhancements although improve the accuracy of conventional films, more false positives are made and thus these techniques would not be recommended. This would give rise to the usage of digital radiographs especially with digital contrast enhancement (Wenzel *et al.*, 1992).

A systematic review by Wenzel in (2004a) and (1995) found that radiographic examinations in most laboratory studies are highly in comparison with visual examinations with sensitivity ranges from 50-70%. On the other hand, in another systematic review by Bader in (2002) found a wider range of sensitivities i.e. 20%-75%, although associated with a moderate range of specificities. He found that the pattern of the results for digital methods was similar to film-based systems but with higher specificity values.

Most investigators suggest that there is a very small probability of cavitation where the radiolucent lesion is within enamel (Pitts and Rimmer, 1992; Akpata *et al.*, 1996). Hintze *et al,* (1998) examined 53 dental and dental hygiene students, with a mean age of 24 years, and found that 8% of lesions with a radiolucency within the inner half of enamel were cavitated.

All of these previous studies have concluded that radiolucency in the outer half of dentine is highly indicative of associated tooth surface cavitation. However, it is not an absolute predictor of cavitation and, radiographs should, therefore be used in conjunction with other detection methods in decision-making about the need for restorative intervention versus preventive only measures. With respect to a radiolucency in the inner half of dentine, studies have almost always, shown this to indicate cavitation (Nielsen *et al.*, 1996).

It could be argued that BW radiographs are able to detect more proximal lesions than occlusal lesions especially in enamel. This would be beneficial as adjunctive method for visual examination of proximal surfaces. This was supported by a study conducted by Hopcraft and Morgan in (2005) who examined 879 adults from a low caries risk population to assess the value of radiographs compared to clinical examination for the diagnosis of proximal caries in permanent teeth. They found that bitewing radiographs provided a significant additional diagnostic yield of 204-336% with two thirds of proximal lesions detected on bitewing radiographs.

Digital Bitewing radiographs Digital imaging was introduced into oral radiology at the end of the 1980s. There are several digital systems available in the market to substitute the conventional films including direct and indirect imaging systems. The most common direct imaging systems using a solid state sensors are Charge-Coupled Device (CCD) and Complementary Metal Oxide Semiconductor (CMOS) (Shearer *et al.*, 1990; Farman, 1995; Borg, 1999). The indirect digital imaging systems introduced, in the mid-1990s use mainly Photostimuable Phosphor (PSP) or Known as storage phosphor plates (Kamburoğlu *et al.*, 2010c; Kamburoglu *et al.*, 2011). The PSP was presented as an alternative way than using a conventional film in intraoral radiography. An image plate

is placed in the mouth and exposed. The plate is then removed and developed a in a laser scanner and presented on a computer monitor. The sensitivity of this system is higher than for film and the development time is reduced to 30 s. The image plates are comparable to film in terms of size. The plates are reusable. Recently, digital radiographs become more popular in dental practices because images are easily manipulated to improve the contrast, brightness and magnification if needed. The other advantage over conventional BW is the reduced use of chemicals for processing of plain films and mainly involved in smaller radiation doses In addition, the lack of destroyed processing artefacts often associated with conventional films and the possible image quality enhancements would improve the overall quality of images (Wenzel, 1995; Pai and Zimmerman, 2002).

Previous studies showed comparable performance between digital and conventional radiographs for detection of occlusal caries (Young *et al.*, 2009; Hintze *et al.*, 2002; Møystad *et al.*, 1996; Wenzel, 1995).

Contrast is determined by the difference between the darkest area and the lightest area of the image. The contrast may vary depending on the structure that is being analysed; optimum contrast depends on the diagnostic task. For instance, an image with high contrast is better for the diagnosis of proximal caries (Wenzel *et al.*, 2002; Syriopoulos *et al.*, 2000). A digital image is an adjustable image; its contrast and brightness can be changed according to observer's needs. It is recommended that radiographs need to be quite dark with good contrast to provide an accurate level for caries diagnosis (Costa *et al.*, 2001).

It has been shown in the literature that the use of radiographs is more sensitive than clinical inspection for detecting proximal lesions and for occlusal lesions in dentine, for estimating depth of the lesion, and for monitoring lesion behaviour. However, detection

performance for occlusal enamel lesions is lower (Gomez *et al.*, 2013b; Bader *et al.*, 2002). Furthermore, in occlusal surfaces, the contribution of the radiographs seems to be minimal (Chong *et al.*, 2003). Moreover, radiography cannot distinguish between active and arrested lesions and sometimes between non-cavitated and cavitated lesions (Wenzel, 2004a). Digital radiographs seem to be safer to the patient, requiring less irradiation and can be stored electronically. However, they have only a potential of 256 grey levels compared to conventional radiographs containing millions of grey levels; this would suggest that digital radiographs would have lower resolution; sensitivity and specificity have been found to be lower than traditional radiographs. However, algorithms to enhance grey scales can be applied increasing its sensitivity and specificity (Pretty, 2006).

A number of earlier studies have compared image quality with different solid-state and PSP systems with conventional film. Most of these studies found that digital images are comparable with conventional images and it is recommended as a satisfactory method to capture x-ray images (Kashima, 1995; Lim *et al.*, 1996; Huda *et al.*, 1997).

From validation point of view, it has been found that lesion depth is usually underestimated in the radiographic examination when compared with lesion depth measurements on the histological sections (Jacobsen *et al.*, 2004; Young and Featherstone, 2005).

Digital panoramic x-ray (DPT)

Panoramic x-rays are the most frequently used extra-oral films in clinical practice despite their low diagnostic accuracy for caries detection (Bader and Shugars, 1993). It has been reported in the past, that DPT is taken by dentists for diagnosis of dental caries (Osman

et al., 1986), in individuals with a heavily restored dentition (Rushton *et al.*, 1999),and routinely for every new adult patient attending dental practice .

Even though it has been widely accepted, it still carries several down sides such as overlapping of proximal surfaces mainly on premolars and superimposition of head and neck structures (Rushton *et al.*, 1999; Tronje *et al.*, 1981; Wyatt *et al.*, 1995).Very little have been published regarding the usage of digital Panoramic x-ray in caries diagnosis for occlusal lesions (Kaeppler *et al.*, 2007; Ramesh *et al.*, 2001). A previous study by Clifton *et al.*, (1998) aimed to evaluate three extra-oral radiographic imaging modalities as alternatives to conventional intra-oral for the detection of occlusal and proximal caries. The found that Panoramic imaging cannot be used soly for occlusal caries detection due to the fairly wide image layer (15-30 mm) which could superimpose the coronal anatomy on the region of diagnostic interest.

The selection criteria produced in 1998 by the Faculty of General Dental Practitioners (Faculty of General Dental Practitioners (UK) The Royal College of Surgeons of England. Selection Criteria for Dental Radiography. 1998.) clearly exclude the routine use of panoramic radiographs for new patients and for the heavily restored dentition, and report that the image quality of panoramic radiographs is inferior to that of intra-oral bitewing radiographs.

Few studies compared panoramic x-ray with bitewing radiographs for occlusal and proximal caries detection. All included studies found bitewing radiographs to be superior to panoramic radiographs in terms of accuracy and only two studies found any evidence to the contrary i.e. (Ramesh *et al.*, 2001; Thomas *et al.*, 2001). Both studies were clinical and used a new digital panoramic systems. The reference standard was not robust i.e.

consensus scores. However, there was a high degree of variability between observers in both studies, which may bring the quality of the study into question.

1.2.3 Cone Beam Computed Tomography (CBCT):

Over the last three decades, there has been a revolution in the medical CT technology in parallel with the invention of more compact machines such as cone beam CT which can be used in the dental practice. Cone-beam computed tomography or (CBCT) systems had been introduced in the dental use in 1990s which is composed simply of a 2D x-ray detector and a cone-shaped x-ray beam to provide isotropic 3 D images at different resolutions(Scarfe and Farman, 2008). CBCT provides a volumetric x-ray scan of the patient's head that can be visualized in a variety of ways such, (i) as multi-planar reformatted (MPR) slices i.e. sagittal, coronal and axial sections at different section slice thicknesses and intervals, (ii) reformatted two dimensional panoramic view, and (iii) volumetric rendering (Shahidi *et al.*, 2015). CBCT is capable of providing sub-millimetre resolution of images with acceptable levels of contrast , short scanning times (10–50 seconds) and radiation dosages reportedly up to 15 times lower than those of conventional CT scans (Tyndall and Rathore, 2008).

A recent review has shown that effective dose (ICRP 2007) from a standard dental protocol scan with a 64-slice multi-detector CT (MDCT) unit is 830 µSv which is 1.5 to 12.3 times greater than comparable medium- field of view dental CBCT scans (10 to 650µSv). This range depends on the field of view required and the settings of the available CBCT machines in the market. However, the effective dose for intraoral bitewing radiograph is in the range of (5 to 8µSv) (Ludlow and Ivanovic, 2008; Wenzel, 2014).

The use of CBCT in dentistry, as a three dimensional imaging modality, is becoming increasingly popular. Its potential to visualize minute changes in both the teeth and the surrounding structures is making it the modality of choice for better visualization of teeth and surrounding structures. For example, it can help in the planning and assessment of root canal treatment, implant placement and periodontal defects (Connert *et al.*, 2014; Verhamme *et al.*, 2014). However, as CBCT is a new radiographic modality, its accuracy had to be compared with an existing well-established system i.e. intra- and extra-oral conventional and digital radiograph. However, very little has been published on the ability of this technology to detect caries (Haiter-Neto *et al.*, 2008; Tyndall and Rathore, 2008). A summary of the most relevant studies concerning the diagnostic accuracy of CBCT for the detection of occlusal and proximal lesions on permanent teeth is presented in Table 1.1.

Akdeniz *et al*, (2006) compared the 3DX-Accuitomo CBCT, Digora film (PSP) and conventional film for measuring depth of proximal caries lesions. The authors found that CBCT images provided more accurate lesion depth estimates with less variation when compared with measurements performed on the sections of the tooth than did the intraoral images and therefore suggested that CBCT has the potential for monitoring small caries lesions. However, this finding needs further investigation with another robust radiographic modality i.e. Micro-CT.

In an editorial by Farman, the principle of ALARA (As Low As Reasonably Achievable) is still fundamental for diagnostic radiology and CBCT procedures should be reserved for selected cases (Farman, 2005) .

Many authors (Tsuchida *et al.*, 2007; Şenel *et al.*, 2010; Qu *et al.*, 2011; Park *et al.*, 2011) have indicated that CBCT systems offer no significant improvement on the diagnosis of

dental caries when compared with intraoral radiographic techniques. However, some authors (Akdeniz *et al.*, 2006; Young *et al.*, 2009; Wenzel *et al.*, 2013) have reported improved caries detection using CBCT systems compared with intraoral radiography. In conclusion, there is agreement in the literature that there is no difference between all radiographic modalities (2D and 3D) in detecting early occlusal lesions. However, CBCT performed better on detecting deep dentinal lesions on occlusal and proximal surfaces. Despite of this, the usefulness of CBCT for detection noncavitated lesion in the literature is still is controversial and requires further investigation.

The variation in the results among these studies is related to the difference in the sample size and selection of teeth, the type and settings i.e. exposure time of the machine used and the experience of the examiners (Lazarchik *et al.*, 1995; Krzyzostaniak *et al.*, 2014) One of the inherent disadvantages of CBCT is the lower spatial resolution which is ten times lower than conventional film (Farman *et al.*, 1995; Parks and Williamson, 2002; Kulczyk *et al.*, 2014). This will lead to difficulty in diagnosing early enamel lesions associated with minimal changes in the mineral concentration. Another disadvantage is the beam hardening effects which result in cupping artefacts related to the distortion caused by metallic structures within the teeth i.e. restorations, posts and implants. Other artefacts include streaks and dark bands around the margins of restorations (Katsumata *et al.*, 2006). These factors could induce errors in the interpretation of CBCT images as well as errors caused by patient movement during image acquisition which will leads to reducing the contrast of the images and scattering into the area of interest (Clifton *et al.*, 1998; Ricketts *et al.*, 1995a).

The SEDENTEXCT project in Europe makes recommendations, based on guidelines for the applications of CBCT in dentistry including referral criteria and optimization

strategies. The guidelines developed using previous systematic reviews, involving stakeholders' advice. The main stakeholder was the European Academy of Dental and Maxillofacial Radiology who founded the basic principles for the use of dental CBCT. These recommendations include not using CBCT solely for caries detection due to the higher doses of ionizing radiation involved as compared with intraoral radiography. In addition, CBCT cases should be kept for select cases because it is critical that the potential patient benefits should be balanced against the risk of exposure to ionizing radiation (Ludlow *et al.*, 2003; Farman, 2005).

Author	Surface	CBCT Device	Intra-oral modality	Results
(Akdeniz *et al.*, 2006)	Proximal 41 teeth, *in vitro*	Limited cone beam computed tomography (LCBCT)	Image plate system and F-speed film	CBT better than intra oral radiography in detecting proximal caries
(Tsuchida *et al.*, 2007)	Proximal 50 teeth, *in vitro*	3DX -Accuitomo CBCT	Kodak Insight films	No difference between accuracy in detecting proximal carious lesion
(Haiter-Neto *et al.*, 2008)	Both 100 teeth, *in vitro*	NewTom 3G and 3DX Accuitomo	PSP and Kodak Insight films	NewTom 3G has lower diagnostic accuracy than others
(Tyndall and Rathore, 2008)	Comprehensive review			CBCT systems are better than intraoral systems at detecting carious lesions when no metallic elements present in the oral cavity
(Young *et al.*, 2009)	Both 92 occlusal and 100 proximal surfaces, *in vitro*	3D-Accuitomo	Gendex 1000 X-ray unit	3DX high resolution CBCT better in detecting proximal caries but not occlusal caries when compared with CCD images.

(Şenel *et al.*, 2010)	Proximal 138 teeth, *in vitro*	ILUMA ultra CBCT	Trophy Trex X-ray unit, Progeny Vision DX, Digora Optime	No significant difference between the systems.
(Kayipmaz *et al.*, 2011)	Both 72 teeth, *in vitro*	Kodak 9500 CBCT	Trophy ETX intraoral Xray unit and Digora Optime	Significant difference seen between CBCT and conventional radiography for occlusal caries. No significant difference between systems for proximal caries
(Kamburoğlu *et al.*, 2010c)	Occlusal 130 teeth, *in vitro*	Iluma Ultra CBCT	CCD direct digital intraoral sensor	At all voxel sizes, CBCT is a useful tool for occlusal caries detection
(Qu *et al.*, 2011)	Proximal 50 teeth, *in vitro*	NewTom 9000; Accuitomo 3DX; Kodak 9000 3D; ProMax 3D; and DCT PRO		No significant difference between system
(Charuakkra *et al.*, 2011)	Proximal 120 teeth, *in vitro*	Pax-500ECT, ProMax 3D	F-speed films (Insight Dental Film)	CBCT better than intraoral radiography in detecting secondary caries
(Rathore *et al.*, 2012)	Occlusal 60 teeth, *in vitro*	Sirona Galileos CBCT	Intraoral Planmeca system	CBCT cannot be used solely for occlusal caries
(Wenzel *et al.*, 2013)	Proximal 257 surfaces, *in vitro*	3D Accuitomo FPD80	Digora Toto Digora Optime	CBCT better than intraoral systems in detecting proximal carious lesion
(Sansare *et al.*, 2014)	Proximal 79 surfaces, *in vivo*	Kodak 9000 3D	Conventional film and PSP	CBCT was more accurate in detecting cavitation in proximal surfaces than bitewing radiographs
(Ertaş *et al.*, 2014)	Occlusal 125 teeth, *in vitro*	New Tom 5G CBCT	PSP, Conventional film, direct imaging system	CBCT was better for detecting deep occlusal lesions

(Shahidi *et al.*, 2015)	Simulated occlusal lesions, 80 teeth	New Tom VGI Flex CBCT	PSP	CBCT was better for detecting secondary lesions
(Alomari *et al.*, 2015)	Dentine occlusal lesions, 160 teeth, *in vitro*	Planmeca ProMax 3D Mid	PSP and conventional film	CBCT were able to pick up more dentine lesions

Table1.1 Summary of relevant studies on the diagnostic performance of CBCT for detection of occlusal and proximal caries on permanent teeth.

1.2.4 Fiberoptic Transillumination (FOTI):

Fiberoptic Transillumination (FOTI) is a simple, safe, inexpensive method which may supplement the clinical examination for detection of caries especially on proximal surfaces in every day practice (Davies *et al.*, 2001; Côrtes *et al.*, 2000; Vaarkamp *et al.*, 1997). This method is based on passing a narrow beam of bright white with intensive light source (High-Intensity LED light source) using a probe tip of appropriate size. Light is directed across areas of contact between proximal surfaces from buccal and lingual aspects thus transilluminating the surface. The light passes into the cervical tooth structure and then radiates occlusally. The light is scattered more in demineralised enamel than in sound enamel thus the lesion appears dark against a light background. Caries appeared as a dark shadow on the occlusal surface. Also carious dentine appears orange brown or grey underneath enamel (Neuhaus *et al.*, 2009a).

Most of early studies are focused more on detection of proximal lesions. Stephan *et al*, in (1987) compared between FOTI, visual and radiographic caries detection methods during a 3-yr clinical dentifrice trial which initially involved 3003 children (52,000 proximal surfaces). When FOTI data were compared to radiographic data, FOTI could only detect 17-48% of radiographic lesions. They concluded that FOTI may need further investigations to be a substitute for bitewing radiography. Peers in (1993) suggested that proper training is needed to achieve comparable results with BW radiographs for

proximal lesion detection as most examiners found difficulty to classify the shadows within teeth consistently. Before this study, Holt and Azevedo in (1989) found that FOTI were able to detect more dentinal lesions than enamel lesions.

FOTI has been used for detection of occlusal lesions as an adjunctive method in combination with visual examination. The performance of FOTI for detection of dentinal lesions on the occlusal surface was better than detection of enamel lesions in comparison with visual and intraoral radiographic examinations for occlusal surfaces (Côrtes *et al.*, 2003; Wenzel *et al.*, 1992). Although FOTI is a safe and non-invasive method to be used in daily clinical practice, it is not capable of monitoring caries lesions as this method is highly subjective and there is lack of well-defined classification criteria based on the histological depth of caries (Neuhaus *et al.*, 2009a).

Bader *et al.,* (2002), in a systematic review on the performance of different methods for caries detection, found that, although this method achieved moderate to high specificity values 0.85-1.00, there was a wide range of sensitivity 0.04-0.74 indicating a high level of variability and inconsistency among methods (Ashley *et al.*, 1998; Côrtes *et al.*, 2000; Côrtes *et al.*, 2003). These findings were supported with a recent systematic review by Gomez in (2013a), who reported findings for non-cavitated carious lesions (NCCLs) using FOTI with sensitivity values ranging from 0.21 to 0.96, and specificity values ranging from 0.74 to 0.88. However, FOTI performed better on picking up deep dentinal lesions on occlusal and proximal surfaces.

It has been noted in some studies that FOTI when used as solely subjective method suffered from considerable intra- and inter-observer variation, showing that the use of this technique leads to a high level of intra- and inter-examiner variability (Stookey *et al.*, 1999; Verdonschot *et al.*, 1992; Sidi and Naylor, 1988).

Based on the results of previous studies, it could be argued that FOTI could be an aid for visual examination for detection of deep lesions (Wenzel *et al.*, 1992). However, concerns were raised regarding the sensitivity of such method especially in the presence of appropriate radiographs. Further investigations are required to determine the validity of FOTI for detection of non-cavitated lesions on occlusal and proximal surfaces.

1.2.5 Light Induced Fluorescence (DIAGNOdent):

Fluorescence is based on two wavelengths. The excitation wavelength which is absorbed by the tissue and emitted at a longer wavelength called emission wavelength. The dental tissues possess such characteristics and this is called auto-fluorescence. When a tooth is exposed to red light , the light penetrates the enamel deeply into dentine, and back scattered light of a longer wavelength is measured by a detector to measure the fluorescence of carious dentine (Hibst *et al.*, 2001). Fluorescence investigations showed that when the tooth is excited by red light at wavelength 655nm, considerable differentiation was found between sound and carious dentine (Featherstone and Hibst, 2001; Hibst and Paulus, 1999). On the basis of this finding, the DIAGNOdent, a laser fluorescence-based device was introduced in 1998. It was designed as a portable, handheld, pen-like device emitting light from a diode laser that could can differentiate between sound and carious tooth tissue (König *et al.*, 1998). The unit gave a digital numeric read-out (0-99) to indicate the amount of fluorescence transmitted back to the detector in the presence of caries. The amount of detected fluorescence was expressed as a digital number from 1-99 (Hibst and Paulus, 1999). The DIAGNOdent did not produce an image of the tooth; instead it displayed a numerical value on two LED displays. The first displayed a current reading while the second displayed a peak reading for that particular measurement. A calibration kit was supplied with the system, and the device

was calibrated using sound enamel on every tooth so that the zero value of fluorescence could be obtained and subtracted (Lussi *et al.*, 1999; Stookey *et al.*, 1999; Shi *et al.*, 2001; Rodrigues *et al.*, 2009).

Initial evaluations of the device in relation to validity and reproducibility of DIAGNOdent for the detection of caries on different tooth surfaces has been investigated suggesting that it may be a promising tool for clinical use (Lussi *et al.*, 1999; Shi *et al.*, 2001; Pinelli *et al.*, 2002).

Lussi *et al,* in (2001) completed an *in vivo* study where they tested the DIAGNOdent in order to determine if the device should be recommended for use in the dental office. Based on the results of this study the following guidelines for clinical use of the DIAGNOdent were recommended in order to minimize the number of possible false positive readings: 0-13, no active care advised; 14-20, preventive care advised; 21-29, preventive or operative care advised depending on patients risk assessment and for Values >30, operative care is intended.

DIAGNOdent was useful in investigating the effectiveness and monitoring caries-preventive effects of a fluoride and quantifying smooth-surface incipient caries lesions in primary teeth based on measuring changes in the readings at different periods (Gokalp and Başeren, 2005; Mendes and Nicolau, 2004; Sköld-Larsson *et al.*, 2004).

There has been some debate over what exactly the DIAGNOdent is measuring; it is not affected by the intrinsic changes within the enamel structure i.e. demineralisation and remineralisation. Instead the system is thought to measure the degree of bacterial activity. This is supported by the fact that the excitation wavelength is suitable for inducing fluorescence from bacterial porphyrins; a by-product of the metabolism of bacterial. This by-product provokes fluorescence and the intensity of the emitted light is related to the

size of the carious lesion (Verdonschot and van der Veen, 2002; Ástvaldsdóttir *et al.*, 2010).

However, this device, like many novel caries detection devices, requires teeth to be clean and dry. In general, *in vivo* studies of LF indicate moderate to high sensitivity and lower specificity. In addition, there is an increased likelihood of false-positive readings due to stain and plaque, which may require a different set of cut-offs to compensate for stain and/or discoloration when using them. Nevertheless these underlying factors, the desire among authors to recommend the LF method as a supplementary method for caries detection (Shi *et al.*, 2001; Côrtes *et al.*, 2003).

In a previous systematic review conducted by Bader and Shugars (2004), the authors found that, for dentinal caries, the DIAGNOdent device performed well, although there was a great deal of heterogeneity in the studies and they were all undertaken *in vitro*. Their conclusion was therefore that there was insufficient evidence to support the use of the device as a principle means of caries detection in clinical practice.

Based on this findings, in 2006, new device known as the DIAGNOdent pen (LF pen, Kavo, Biberach, Germany), was introduced in the market. The DIAGNOdent pen was a less bulky, flexible, and cordless mobile instrument with differently shaped tips for different purposes. The mechanism behind the principle function of the DIAGNOdent pen is the same as for the conventional DIAGNOdent system. However, the propagation of both the excitation light and fluorescence light occurs in the same single solid fibre tip in opposite directions. This is in contrast to the first device where excitation light is transported through a central fibre while fluorescence light is collected from hard tissue through additional fibres which are concentrically arranged around the central fibre (Lussi and Hellwig, 2006; Lussi *et al.*, 2006a). The device has two different fibre tips, a conical

tip, with a diameter of 0.7 mm at the measurement site for use on proximal surfaces and a cylindrical tip, with a diameter of 1.1 mm for use on occlusal and smooth surfaces. The conical tip can rotate around its long axis to allow placement of the probe on the mesial and distal surfaces at the oral and facial sides in anterior and posterior teeth. A red point on the side of a metal bezel indicates the light direction.

The cut-off values of the new device were found to be slightly different to those of the original device. However, compared to the old device, it has been shown to have the same validity and reproducibility for the detection of occlusal caries (Kühnisch *et al.*, 2007).

The new LF device (DIAGNOdent pen, Kavo, Biberach) was first validated for the detection of proximal caries by (Lussi *et al.*, 2006a). They conducted an *in vitro* study in which they used 75 permanent molars, frozen at -20°C until use. Although, the investigators used frozen teeth in their study, which have been shown to have stable fluorescence values and hence stable cut-off values, the cut-off values from this study cannot be applied clinically as it was not an *in vivo* study. Later, a randomised clinical trial by Huth *et al,* in (2010) was conducted to clinically validate the previous results of the Lussi *et al.,* (2006). The investigators concluded that the new device may be used as an additional diagnostic tool for detection of proximal caries in permanent teeth. Although the same cut-off values as the *in-vitro* study were proposed, the limitations of the *in-vivo* study make it difficult to confirm the clinical application of these values as there was no true histological examination, which is the gold standard for the validation of results.

Recent systematic reviews on the performance of fluorescence-based devices by Neuhaus *et al,* in (2009b) and Gimenez *et al.,* in (2013c) found a tendency for the device to achieve higher specificity than sensitivity, except for when the dentine threshold was observed

for caries detection. The main issue of low specificities at the dentine threshold is the potential over prescription of operative treatment. They concluded that baseline readings of sound tooth structure need to be taken properly to allow subtraction of the autofluorescence later by the device. Also, it performed better in detecting deep enamel and dentine lesions on occlusal surfaces than proximal surfaces.

In general, a number of factors have been shown to influence DIAGNOdent measurements and could result in higher false positives cases. First, the presence of deposits such as fissure sealants as stain, plaque, calculus, toothpaste or prophylactic paste which may give rise to false positive results. Thus, some investigators suggested that thorough cleaning is an essential prerequisite to accurately diagnose caries using the DIAGNOdent device (Deery *et al.*, 2006; Bamzahim *et al.*, 2005; Bamzahim *et al.*, 2004; Lussi *et al.*, 2005; Lussi and Reich, 2005). However, this effect seems to be limited to occlusal surfaces as it has no effect on the performance of the LF pen on non-cleaned proximal surfaces (Bittar *et al.*, 2012). In the case of *in-vitro* studies, the storage solution, such as chloramines, formalin or thymol have been found to cause significant reduction in fluorescence of extracted teeth stored in these solutions. In contrast, teeth which have been frozen at -20 ℃ without a storage solution showed no significant change in fluorescence over 2 years (Francescut *et al.*, 2006).

1.2.6 Electrical Conductance-Based Methods:

The current in all electrical devices to detect caries involves the application of a certain voltage. In the case of Direct Current (DC) measurements, a constant voltage is applied across the tooth tissue. However, by varying frequency this voltage and using an Alternating Current (AC) much broader information on the electrical properties of the tissue, compared to DC measurements, can be achieved (Huysmans *et al.*, 1996). The

properties of a normal tooth are such that dentine is more conductive than enamel (Matoorian *et al.*, 1995). It can be concluded that, when affected by caries, tooth structure (enamel or dentine) has an increased porosity resulting in a higher fluid content. The presence of this fluid within the tissue improves electrical conductivity. This change in conductivity can be detected and measured. This quantifiable value can then be used to determine the presence of dental caries. Studies since 1950, have highlighted the potential clinical usefulness of electrical conductance of dental hard tissues over conventional visual examination for detecting the presence and extent of carious lesions (Pincus, 1950; Mumford, 1967). Early work, by Barden in (1976); involved the application of direct current. However, caries detection by the measurement of electrical resistance is hampered by polarization effects when DC or single-low-frequency AC currents are used. Therefore, AC current measurement devices with different frequencies were developed in order to overcome polarisation effect (Mayuzumi, 1964; White *et al.*, 1978) .

Later, in early 80's, several devices had been developed utilizing AC current, but using single, fixed frequencies for experimental convenience. Hence, most of these studies weren't useful as the actual details of such parameters applied hadn't been given in many of the publications.

The Caries Meter-L was developed and used by Matsumoto in (1981) to correlate artificial carious lesion with electrical resistance of teeth. The Caries Meter-L was shown to have sensitivity and specificity of 0.74 and 0.74 for D_1 threshold and 0.96 and 0.62 for D_3 diagnostic threshold for occlusal caries detection (Ricketts *et al.*, 1995b). Another device was invented for occlusal caries detection called Electronic Caries Monitor (ECM) which employed a single, fixed-frequency alternating current which attempted to measure the "bulk resistance" of tooth tissue. The ECM probe was directly applied to the site in

question, which was measured over a five second measurement period. It was concluded that the ECM had very good reproducibility, even in the hands of an inexperienced operator. Despite its repeatability, having the lowest overall specificity in both enamel and dentin lesions means ECM is most likely to indicate operative intervention on sound surfaces (Ricketts *et al.*, 1997; Huysmans *et al.*, 1998; Ashley *et al.*, 1998).

A systematic review by Bader *et al.*, (2002) investigated the performance of detection methods used for caries detection. They found out that the variation in specificity was more pronounced than sensitivity using several different electrical conductance devices i.e. ECM, Vanguard and Caries Meter-L. However, the variation in sensitivity and specificity was narrower among electrical conductance device studies compared with other methods for caries detection and had a range of 0.58 to 0.97.

Actual conductance measurements depend on the conditions under which the electrical measurements were obtained. Variations in the results using different electrical methods under various conditions are due to the impact of several factors affecting the conductivity of dental tissues. For instance, post-eruptive maturation or age of a tooth (Schulte *et al.*, 1999) and enamel thickness (Hoppenbrouwers *et al.*, 1986) can all affect the electrical conductance of teeth. In addition, the surface area of site, the type of conducting medium (Mosahebi and Ricketts, 2002) and the variation in the temperature of the mouth (Huysmons *et al.*, 2000) should be taken into account when performing such experiments *in vivo* or *in vitro*. In general, two different methods are normally used when measuring teeth: Surface-specific – where the entire occlusal surface is covered in conducting medium or; Site-specific – where the electrode makes contact with a single point on the tooth surface. These different methods may produce entirely different resistance measurements on the same tooth (Rock and Kidd, 1988; Verdonschot *et al.*, 1992).

AC impedance spectroscopy Impedance spectroscopy is the resistance measurements obtained from application of sinusoidal currant of varying voltage over a wide range of frequencies (Scholberg *et al.*, 1987). It had been used to determine the electrical properties of sound and carious tooth structure using equivalent circuits resembling the structure of the dental tissue. As stated earlier, ECM relies on fixed frequency alternating current to measure bulk resistance of the tooth, whereas Electrical Impedance Spectroscopy (EIS) used to determine the electrical properties of the tooth under different alternating frequencies. One of the advantages of AC impedance spectroscopy is that, by employing alternating current, polarisation effects are eliminated and various parameters associated with anatomical differences are determined more accurately , and consequently the conductive properties of teeth (Eldarrat *et al.*, 2004; Longbottom and Huysmans, 2004).

The first attempt to investigate AC current at alternating frequencies on teeth was carried out by Kumasaki in (1975), he was able to distinguish between sound teeth and those with shallow or deep dentinal caries. Following this, Levinkind *et al,* in (1990) demonstrated that it was possible to distinguish between bovine, human deciduous and human permanent enamel using ac impedance spectroscopy.

Most of the early devices which rely on impedance spectroscopy are not applicable for clinical use as they are large in weight and size which will hinder their mobility. Another reason, is the necessity to combine several instruments in order to obtain impedance measurements. In view of the above, a fully embedded handheld commercial diagnostic device was developed to overcome such constraints and is capable to generate and analyse the impedance spectrum.

A clinically-useable device, based on AC impedance spectroscopy, was developed by a Dundee University spin out company and marketed commercially as CarieScan PRO and was introduced to the market in 2008. It is a handheld device for dental caries detection implementing an impedance current system with multiple measurement channels that operates in the frequency domain with range of 100 Hz to 20 KHz. A full description of the hardware architecture and design software has been described in detail by (Guimerà *et al.*, 2008).

This device applies a frequency sweep to the tooth and analyses each spectrum in <1s. The system is intended to be run autonomously. It includes a 4-channel multiplexer and is capable of measuring impedances from 10 kΩ to 10 MΩ across a frequency range of 100 Hz to 100 kHz with a maximum error of 5%.

The disposable sensor used to measure a selected investigating site on the occlusal surface is prepared from strands of surgical grade stainless-steel (50 µm diameter). The resulting 'tuft' is flexible and conforms to the contact surface.

The sensor is applied to a tooth surface after it is properly dried for 5 seconds. The probe tip of the device remains on the tooth surface for about 3-5 seconds. A score ranging from 0 – 100 will be generated. Red, Yellow, and Green LED pyramids are illuminated on the device to correspond with the numerical score. As one would expect the green pyramid can be seen with scores from 0-50 and indicates sound tooth structure. The yellow pyramid is displayed with scores from 51-90 indicating a likelihood of caries into enamel, and red pyramid is displayed with scores from 91-100 indicating a likelihood of caries into dentine (Table 2.1). The manufacturer of this device claims that this new approach to caries detection is highly successful, resulting in a sensitivity of approximately 95% and a specificity of approximately 93%.

Most of the literature available for this device was found as abstracts which had been submitted to journals regarding the performance of this new device in comparison to other caries detection methods. Initial reports were encouraging. This device could be useful in monitoring serial changes in the caries status of teeth due to its high reproducibility on different occasions and had a superior detection performance compared to other techniques the D_1 threshold. Only a few publications have reported the performance of CarieScan PRO for occlusal caries detection under *in vitro* and *in vivo* conditions on permanent and primary teeth (Mortensen *et al.*, 2014; Melo *et al.*, 2015; Teo *et al.*, 2014). The literature is controversial regarding the performance of this new device in comparison with conventional and novel caries detection methods and so, further *in vivo* and *in vitro* studies of permanent occlusal surfaces are needed to mirror the clinical situation.

1.3 The Development of a Gold Standard for Validation of Caries Detection Methods

Validity, by definition, reflects whether a test actually measures what it is purported to measure. It is logical therefore that the validation method or "gold standard" needs to be more accurate than the detection method. Furthermore the gold standard should be reproducible. It should also reflect the patho-anatomical appearance of caries it is intended to measure and also be independent from the detection method to be validated (Wenzel and Hintze, 1999). However, in some cases it is difficult to fulfil all of these criteria. In any case, the outcome of any diagnostic test evaluation is dependent on the method of validation used (Beck and Shultz, 1986). Ten Bosch in (2003) has subdivided validity into the following areas:

- **Construct validity** to what extent does the measurement correspond to what is generally known about the lesion;
- **Content validity** does the measurement adequately describe what is being described about the lesion;
- **Criterion validity** do changes in the lesion correlate with changes in external criteria. For example does lesion severity correlate with a change in some properties of the lesion. Criterion validity further sub-divided into
    - **Predictive validity** are methods valid to determine lesion behaviour from a single or multiple observations and ;
    - **Concurrent validity** are methods applicable to caries in vitro, in situ or in vivo on a similar scale to caries, for example categorical or continuous.

Ten Bosch and Angmar-Månsson in (1991) described generally accepted validation methods used for caries detection which include transverse microradiography (TMR), polarised light microscopy (PLM) and cross-sectional microhardness measurements (CSMH). These methods were applicable for changes in the mineral content of lesions in enamel.

Even though such techniques are generally accepted there are still large variations in protocols for the same validation method and large variations in agreement for the same methods performed by different examiners (Huysmans and Longbottom, 2004; Ten Cate JM *et al.*, 2000).

For the validation of caries detection methods, different approaches have been used. Direct visual examination after tooth separation has been used for the validation of radiographic examination (Deery *et al.*, 2000; Ekstrand *et al.*, 1997). However, using visual examination as a ‘gold standard’ does not fulfil some of the criteria, since it has

been shown that visual examination is not reproducible and is unable to determine lesion depth (Hintze *et al.*, 1998; Eggertsson *et al.*, 1999; Hintze and Wenzel, 2003). Operative confirmation of caries has been described for *in vivo* studies (Verdonschot *et al.*, 1992) but ethically these methods preclude examination of teeth thought to be sound. Combinations of several clinical detection methods have also been used to validate a particular detection method (King and Shaw, 1979) but such experiments are complex, as the validation methods themselves can be inter-dependent on each other.

Another method for validation of caries detection methods has been radiography (Wenzel *et al.*, 1991a). Radiography has also failed as a 'gold standard' since it is also poorly reproducible (Hintze and Wenzel, 2003). However, there are several forms of radiography and not all are equally acceptable as a reference standard. Wenzel and Hintze (1999) pointed out that the routine clinical use of radiography for caries detection did not fulfil two out of the three criteria for a robust reference standard. It has been suggested that X-ray microtomography (Micro-CT) may be a suitable form of radiography to act as a validation method for evaluation of mineral loss occurring as a result of the caries process (Huysmans and Longbottom, 2004). This method will be discussed in detail in the following section.

A number of histological validation methods have been described for examination of section cut of teeth, these include polarized light microscopy and microradiography (Mileman and van der Weele, 1990; Ekstrand *et al.*, 1998). Both methods required sections to be ground to approximately 100 microns thickness, but such thin sections may miss the area representing the deepest aspect of the lesion or the whole section may be destroyed during cutting (Huysmans and Longbottom, 2004). Thicker sections, above 700

microns, were may also be made and viewed under stereomicroscope (Wenzel *et al.*, 1990) or radiographed and analysed using a measuring grid (Penning *et al.*, 1992).

Examination of a hemisection through the investigation sites on occlusal surface has been used for validation. However, this method has been shown to be less accurate than serial sectioning (Ricketts *et al.*, 1994; Deery *et al.*, 1995; Hintze and Wenzel, 2003).

Tooth grinding has also been employed to facilitate histological examination of caries lesions (Lussi *et al.*, 2006a; Francescut *et al.*, 2006; Neuhaus *et al.*, 2010). In these studies, teeth were ground longitudinally, under constant water, on a polishing machine with silicone carbide paper of different series of grain size.

However, it is generally accepted that one of the most accurate assessments of a detection method is provided by microscopic examination of histologically serial sections under *in vitro* and *ex vivo* conditions (Downer, 1989; Huysmans and Longbottom, 2004; Rock and Kidd, 1988). Most of the studies using serial sections have been undertaken under laboratory conditions and, although it is more convenient, care must be taken to reproduce and simulate the clinical variables.

Serial sectioning of teeth to slices of a thickness of 250-300µm followed by microscopic examination at a magnification of x16-40, has been used to validate the diagnostic performance of different methods for the detection of occlusal caries and proximal caries in permanent and primary teeth (Braga *et al.*, 2009a; Deery *et al.*, 2006; Aljehani *et al.*, 2007).

A number of protocols have been used for histological scoring of the presence and extension of caries. The most commonly employed criteria are those proposed by Downer (Downer, 1975). These criteria use the enamel-dentine junction as an important land mark

between enamel and dentine caries in the assumption that once caries reaches the EDJ it spreads laterally and undermines the enamel surface (see Table 3.1). In (1997), Ekstrand and co-workers developed a more detailed histological scoring system (see Table 3.2). This system combined deep enamel caries with initial dentine demineralisation in score 2 based on the close relationship that was found between enamel caries and reactions in underlying dentine on proximal lesions (Bjørndal and Thylstrup, 1995) and on occlusal lesions (Ekstrand *et al.*, 1995).

1.3.1 Micro-ComputedTomograpy

In the last 2-3 decades, there has been an increasing demand for non-destructive techniques that are capable to quantifying the mineral loss in hard tooth structures. Ten Bosch and Angmar-Månsson in (1991) carried out a detailed literature review of quantitative methods used to determine the mineral content in dental hard tissues and recommended the development of a non-destructive radiological method to measure the mineral within a whole tooth. Micro-CT system has been introduced to the market in the last decade and is considered a novel digital technology that shows great promise for detecting subtle changes within structures. It can create three dimensional images with higher resolution in the range of 5-50μm by using micro-focal, spot x-ray sources and higher resolution two-dimensional detectors (Swain and Xue, 2009).

In 1972 (Hounsfield) invented x-ray computed tomography allowing internal structures of an object to be viewed in three dimensions. Whilst Medical CT allows imaging of larger scale structures within human body , Micro-CT is a miniaturized version which was developed by Elliot and Dover in (1982) which showed a great promise for detecting subtle changes within structures.

Basic Principles:

The linear attenuation coefficient (μ) is the possibility that an x-ray photon of monochromatic radiation will interact with the atoms within a material as it passes through a measured thickness. This phenomenon is in the context of Beers law which states that the attenuation is linear to the intensity of radiation and the amount of absorbing atoms within the object (Davis and Wong, 1996; Wellington and Vinegar, 1987).

Another way to express linear attenuation coefficient is the mass attenuation coefficient (MAC) which is independent of the density and can be calculated in a given material from the sum of the MAC of the constituent elements.

$$MAC = \mu / \rho$$

So to convert a MAC to linear attenuation coefficient (μ), we need to multiply it by density ρ of the material.

$$\mu = \left(\frac{\mu}{\rho} \right) * \rho$$

The significance of determining the distribution of the linear attenuation coefficient of an object in a micro-CT image is to achieve the best contrast within an object (Davis *et al.*, 2013).

Development of Micro-CT Scanners:

Since the development of the first micro-CT scanner by Elliot and Dover in 1982, many improvements have taken place in terms of the detectors and the x-ray sources (conventional and synchrotron), in order to improve data acquisition, accuracy and resolution of the images and to overcome the problems associated with radiation such as blurring and beam hardening artefacts (Davis and Elliott, 2006; Davis and Wong, 1996).

The first generation of Micro-CT is the simplest version which is composed of a pin-hole collimator and a single detector. The mounted specimen translates on a stage and rotates along the z-axis by a small amount between projections until a complete set of projections has been taken (Elliott and Dover, 1982) (see Figure 1.2).

One of the main features of this system is the ability to utilize monochromatic radiation, in a narrow beam, to measure accurately the linear attenuation coefficient, especially for objects of known composition based on the mineral concentration of tissues within the object (Ritman, 2004). The major disadvantage of this system is the long time needed for scanning and reconstructing the data which can take weeks and its limited ability to measure only a small number of slices within a lesion (Anderson *et al.*, 1996).

The next generation of Micro-CT machines was based on x-ray fan-beam geometry and linear array detectors, similar to the third generation Medical CT scanners. In this system a whole slice could be projected and recorded at one time. Despite these modifications and improvements, reconstruction of a three dimensional image was still time consuming, required more complicated algorithms for reconstruction of data and there was inefficiency of data acquisition due to the large number of photons wasted during exposure (Axelsson and Danielsson, 1994).

The third generation of Micro-CT was based on using a polychromatic cone beam radiation and a two dimensional area array detector. Such systems allowed the object to be scanned once in order to create a three dimensional images rather than scanning slice at a time as in the earlier versions (Hamba *et al.*, 2012; Wong *et al.*, 2004). Whilst cone beam CT geometry reduces the scanning time, it needs more complicated reconstruction algorithms because of the polychromatic characteristic of radiation used which leads to beam hardening artefacts (Davis and Elliott, 2006).

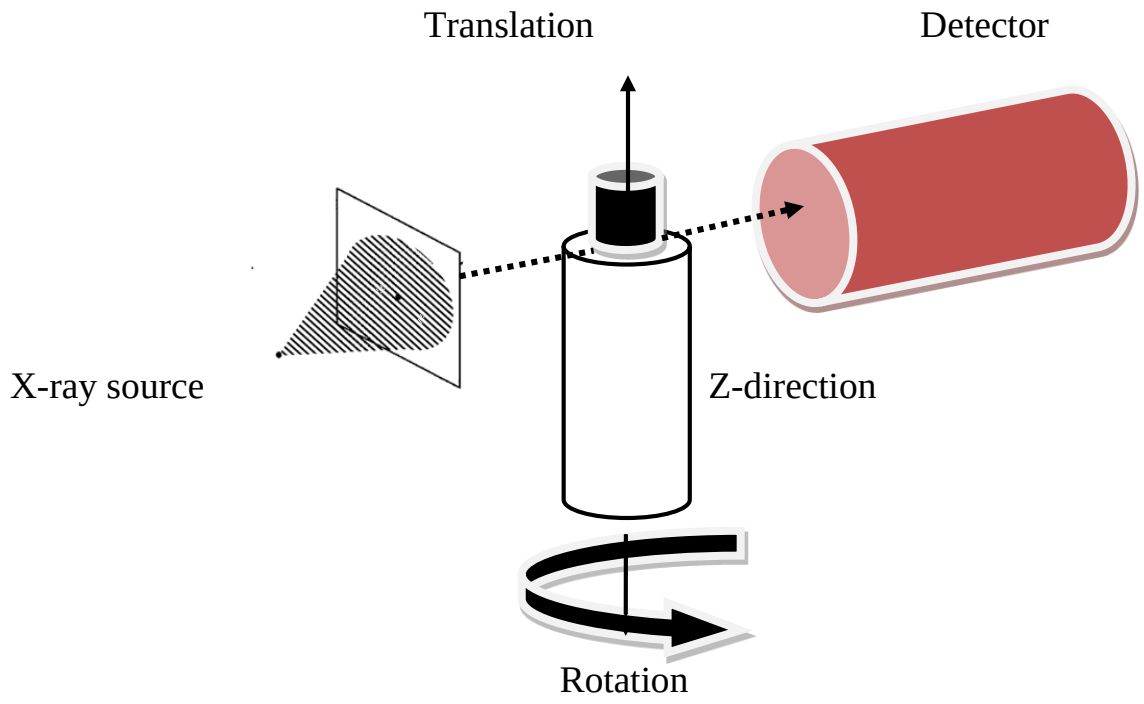


Figure 1.2 Schematic view of pin-hole Micro-CT system.

A complicated fourth generation of Micro-CT (MuCAT system) has been developed to overcome the problem of ring artefacts in the reconstructed images which is caused by differences in the insensitivities of the individual detector elements. This system uses the method of time delay integration in the charged coupled detectors which requires longer exposure time and enhances the signal-to-noise ratio, which in turns improves the accuracy of images (Ahmed *et al.*, 2012; Cochrane *et al.*, 2012).

The most common problems associated with Micro-CT images, created by cone beam scanners using polychromatic x-ray sources, are beam hardening and ring artefacts which can influence the quality of the images especially in case of quantitative analysis.

Beam hardening phenomenon is associated with polychromatic radiation where low-energy photons are generally attenuated more than high-energy photons. Non-uniform attenuation of polychromatic radiation results in the selective absorption of soft x-rays while the hard x-rays are more penetrating or hardened (Davis and Elliott, 2006). This

will cause artefacts which appear as cupping due to the increasing underestimation of linear attenuation coefficient towards the centre of the specimen or in the form of dark bands or streaks between dense objects in the image. The amount of beam hardening depends on the initial x-ray spectrum as well as on the composition and thickness of the material or tissue traversed. Beam hardening can be minimized by using filtration, calibration correction, and beam hardening correction software (Dowker *et al.*, 2003; Brabant *et al.*, 2012).

Ring artefacts are caused by imperfect detector elements, which significantly under- or overestimated attenuation values of reconstructed images thus prevent a proper diagnosis of structures of interest (Davis *et al.*, 2013).

These discrepancies are noted in the reconstructed image as concentric ring-like artefacts which are superimposed onto the initial image and disturb the diagnostic quality of the images. Moreover, it will increase noise and affect homogeneity of Micro-CT images which can result into a reduction of low-contrast resolution.

One way to reduce ring artefacts is known as flat-field correction or by moving the detector array during data acquisition to hamper the effect from varying responses of different detector elements. Also, the artefacts can be eliminated by filtering the images (Riess *et al.*, 2004; Sijbers and Postnov, 2004).

Applications of Micro-CT Technology in Dentistry:

Although Micro-CT was invented in 1982, it is only during the last few years that it has started to gain importance in dental research. Moreover, very few Dental Schools have tested this technology, to benefit from the potential features in terms of improved resolution and using non-destructive methods to achieve higher quality, sensitivity and accuracy of measurements (Swain and Xue, 2009).

Micro-CT has shown to be a useful tool in the quantitative analysis in experimental Endodontology. For example, it has been used to accurately determine the surface area and volume changes within the root canal system before and after instrumentation (Abdo *et al.*, 2012; Vallaeys *et al.*, 2016). Micro-CT was used also in restorative dentistry to determine leakage around restorations (Carrera *et al.*, 2015; Seo *et al.*, 2009). In general, it was useful in studies concerned with dental implants, orthodontics and alveolar bone (Gomes de Oliveira *et al.*, 2012; Paetyangkul *et al.*, 2009; Sennerby *et al.*, 2001).

Micro-CT is a precise technique that allows qualitative and quantitative analysis of trabecular and cortical bone in terms of the degree of mineralization and their mechanical properties which is of great significance in implant dentistry (Davis and Wong, 1996)

Micro-CT in Caries Research:

Initial dental application, such as quantification of mineral changes, started in the early 1990's, when Gao *et al.*, (1993) studied the mineral distribution in enamel rods of permanent teeth before and after demineralization, and after remineralization. Also, it was found that Micro-CT could play a role in understanding the process of remineralization of early carious lesions especially in longitudinal studies as well as in earlier studies using deciduous teeth (Fearne *et al.*, 1994).

Since that time, Micro-CT has been used to study the mineral content of permanent teeth and deciduous teeth. The mineral content of enamel and dentine in human premolars as well as enamel pearls were reported by (Anderson *et al.*, 1996) . The results showed agreement of mineral distribution in the premolars with earlier microradiographic studies and density measurements.

The first experiment conducted on whole teeth was published by Dowker *et al,* in (2003) studying the mineral distribution and the percentage of mineral loss by volume on artificial enamel caries. They found out that the measured mineral concentration of sound enamel varied between 2.6 and 3.1 g_{Hap}.cm–3.

Alternatively, the threshold of mineral loss for natural dentine caries was difficult to define as dentine is a complex composite structure which is composed of 70% wt. inorganic material, 20% wt. organic matrix and 10% wt. water (Frank, 1999; Marshall Jr *et al.,* 1997). Wong *et al.*(2006) used the threshold 1.2 gcm^{-3} based on previous study performed by (Willmott, 2003) . Interestingly, it was found that remineralised dentine is more highly mineralized than sound dentine about 2.25 $g.cm^{-3.}$ This was attributed to the deposited mineral and calcification of dentinal tubules (Kinney *et al.*, 1994).

Micro-CT has been used to determine the mineral density of natural and artificial enamel white spot lesions where pure hydroxyapatite phantoms at different densities were used as calibration standards; whereas for studying the mineral density of dentine, it was suggested that the contribution of water and organic material should be taken into account (Huang *et al.*, 2007; Schwass *et al.*, 2009; Zou *et al.*, 2009).

Micro-CT as Validation Technique:

It has been suggested that Micro-CT may be a suitable form of radiology as a robust reference standard for evaluation of the depth of mineral loss as well as the amount and percentage of mineral lost as a result of caries process (Huysmans and Longbottom, 2004).

However, very few studies have been carried out to compare the images obtained by Micro-CT with the histological sections regarding caries extent or even with other caries diagnostic devices (Mitropoulos *et al.*, 2010).

Micro-CT has been used for validation of the performance of different radiographic modalities i.e. CBCT and bitewing radiographs, DIAGNOdent, and ICDAS on permanent and primary teeth. Most of these studies showed that Micro-CT compared favourably with histology. In addition, Micro-CT has the potential to be a suitable tool for validation of the performance of caries detection methods on occlusal and proximal surfaces of teeth (Kawato *et al.*, 2009; Kamburoğlu *et al.*, 2010a; Taylor *et al.*, 2010; Soviero *et al.*, 2012).

Further studies should be proposed regarding the capability of Micro-CT in terms of qualitative and quantitative analysis of caries lesion i.e. depth and volume; the amount of mineral lost in a lesion and to determine whether Micro-CT has the potential to substitute for conventional histology as a validation method.

In summary, Micro-CT was described as non-destructive method visualization of objects. It does not require specimen preparation, thus there is no loss of tissue or information. It can be viewed in an infinite number of planes without superimposing structures and can be rendered 3-dimensionally making it invaluable for longitudinal studies.

On the other hand, one of the major disadvantages of Micro-CT that it couldn't be used for *in vivo* studies due the higher ionized radiation associated with Micro-CT imaging; some inherent limitations such as noise and image artefacts; and limitation of specimen size.

However, the advantages of using this technology in dental caries research, especially longitudinal studies to monitor remineralisation and /or demineralisation of caries lesions and to determine the effectiveness of preventive measures, would be of great importance and outweigh its disadvantages (Neves *et al.*, 2011; Zhang *et al.*, 2013).

1.4 Statistical Evaluation of a Detection Method

Statistical evaluation of disease detection methods is essential to assess how accurate and reproducible they are under laboratory conditions to provide supportive evidence to be used clinically. It allows comparisons to be made between different detection methods and between examiners. Once the detection method has been developed, the presence and absence of disease needs to be validated. In general, accuracy is defined as "the closeness of agreement between the method outcome and the accepted reference value".

The simplest measure of the validity of a detection method is best illustrated by dichotomisation of results obtained from the method compared to a similarly dichotomised validation method based on cut-off readings/scores at certain thresholds i.e. sound versus enamel and dentine or sound and enamel versus dentine. Four possible outcomes exist. These are true positive (TP), true negative (TN), false positive (FP) and false negative (FN). These outcomes are summarised in Table 1.2, the so-called 2×2 contingency table.

Sensitivity or the true positive is the proportion of disease surfaces correctly detected by the gold standard and can be calculated as: **TP / FN + TP.**

Specificity or true negative is the proportion of sound surfaces correctly detected by the gold standard and can be calculated as: **TN / TN + FP**.

Both sensitivity and specificity can be written as decimals or percentages. They are considered the mostly commonly quoted variables used by studies for verification of detection methods and used in systematic reviews for comparisons among different studies. These two parameters will be used in this thesis to demonstrate the validity of caries detection methods.

Results from validation method "gold standard"	Results from detection method		Total
	Sound	Carious	
Sound	**TN**	**FP**	**TN + FP**
Carious	**FN**	**TP**	**FN + TP**
Total	**TN + FN**	**FP + TP**	

Table 1.2 Contingency table for validation of detection methods.

Accuracy, is an index measures the proportion of all surfaces detected correctly where the total number of true positives and true negatives divided by the total number all decisions. This is dependent on the disease prevalence: **TP + TN / TN + FP + FN + TP.** The horizontal portions in Table 2.1 represent the calculations based on the "gold standard" to determine the accuracy of a detection method. If the table is analysed vertically, the number of correctly carious surfaces out of all positive results of the detection method or the probability of caries detection to be correct is called **positive predictive value** (P / FP + TP). Similarly, the probability that there is no evidence of disease when evaluated by the detection method is called **negative predictive value** (TN / TN + FN).

The likelihood ratio is calculated by dividing the sensitivity by the false positive ratio (1-specificity). Methods with high likelihood ratio are better indicators of disease presence and absence i.e. high specificity and sensitivity.

1.4.1 ROC (receiver operating characteristic) Analysis:

This technique can be viewed as an extension to sensitivity and specificity and is applied to detection methods that are based on continuous and/or ordinal variables. The technique

was originally developed in the early days of RADAR for detection and interpretation of radar signals against a background noise.

An ROC curve is generated by plotting a graph of sensitivity against 1 − specificity for all values of the test (Beck and Shultz, 1986). For analysis of the ROC curve, the greater the area under the curve, the better the method. The maximum area possible is 1 which represents a perfect detection method with no false positive cases. The diagonal line represents the values expected by chance alone i.e. the test produces a false positive result at the same rate as true positive results. It was used firstly in dentistry for validation of radiographic methods base on five- point rating scales also known as "certitude scales" (Nytun *et al.*, 1992). The sensitivity and specificity were calculated at each level of the scale and an ROC curve was plotted.

The ROC technique is also applicable for analysis of diagnostic tests that are based on ordinal (subjective criteria) (Verdonschot *et al.*, 1993b) like those used in dentistry to describe the depth and severity of a lesion. The continuous nature of carious process necessitates that various discrete levels are chosen on this continuum to differentiate between caries and sound surfaces, where sensitivity and specificity are calculated at each cut-off level and ROC curve generated (Verdonschot *et al.*, 1993a).

The area under the ROC curve can be measured to compare performance of two or more detection methods by determining if statistically significant differences occur between the areas under the respective ROC curves. An advantage of using ROC analysis in this way is that it reflects the diagnostic performance more comprehensively than sensitivity and specificity alone, which are determined by only one cut-off point (Kositbowornchai *et al.*, 2004). ROC analysis also distinguishes between the inherent capacities of the examiners to under- and over-estimate readings when interpreting the site under

investigation. In addition, it is useful for establishing optimum cut-off values for detection methods based on a continuous scale. For example, the DIAGNOdent expresses the trade-off between sensitivity and specificity for every possible cut-off value (Beck and Shultz, 1986).

All the variables presented in previous section i.e. sensitivity and specificity, are dependent on the prevalence of disease in the study sample. If the study sample includes high numbers of sound surfaces then this would result in an over estimation of sensitivity. ROC analysis doesn't suffer from such disadvantages (Verdonschot *et al.*, 1993a).

1.4.2 Reproducibility:

Although most of the studies to detect dental caries were performed under standardised conditions, one of the major sources of inconsistency in a detection method is the variation arising from the interpretation by the examiner or rater (known as ***examiner agreement***). If more than one examiner is used then two potential sources of examiner inconsistency are possible: **inter-**and **intra-examiner agreement**. The inter-examiner reproducibility measures the ability of two or more examiners to agree a result from the same subject. Intra-examiner reproducibility is the ability of an examiner to agree a result on different occasions.

In its simplest form, when using ordinal scale, the proportion of decisions made on two separate occasions which perfectly matched can be calculated as the percentage of agreement. However, this doesn't take into account the agreement expected by chance. In addition, it is affected by the number of categories available for scoring. The more values among which an examiner can choose, the more difficult is it to achieve high percent agreement (Birkimer and Brown, 1979).

To overcome this issues, Cohen in (1960) formulated a measure, the kappa statistic, which corrected for the proportion of agreement expected by chance. The formula is:

$$\mathbf{K = (P_o – P_e) / (1 – P_e)}$$

The calculation of kappa is based on the difference between how much agreement is actually present ("observed" agreement P_o) compared to how much agreement would be expected to be present by chance alone ("expected" agreement P_e). It has been suggested by Landis and Koch in (1977) that the closer the kappa value is to one, the greater the agreement between the two assessments. Poor agreement was assessed for kappa below 0.20, fair agreement for kappa between 0.21 and 0.40, moderate agreement for kappa between 0.41 and 0.60, substantial agreement for kappa between 0.61 and 0.80 and strong agreement for kappa > 0.81.

Cohen's kappa takes into account disagreement between the two raters, but not the degree of disagreement and all disagreement is treated equally as total disagreement. Therefore when the categories are ordered, it is preferable to use weighted kappa, and assign different weights to subjects for whom the examiners differ by categories, so that different levels of agreement can contribute to the value of Kappa (Shrout and Fleiss, 1979; Fleiss, 1981). This is suitable for detection methods that are based on categorical data but for methods that produce continuous data, a different approach is required. One method is to set 'cut-off' values that essentially turn continuous data into categorical data, so that the data can be analysed using the above method.

For methods that are based on quantitative assessments with a continuous scale, the degree of association between the readings can be illustrated using scatter plots and the correlation of coefficient can be measured where 0-.19 is "very weak", 0.20-.39 is "weak", 0.40-.59 is "moderate", 0.60-.79 is "strong" and > 0.80 is "very strong".

However, although with higher correlation values, the association will be stronger, it doesn't mean that the two methods agree perfectly. To overcome this issue, intraclass correlation coefficient (ICC) has been designed to assess the reliability of ratings by comparing the variability of different ratings of the same subject to the total variation across all ratings and all subjects. Lin's intra-class correlation coefficient (ICC) was used to assess the inter- and intra-examiner reproducibility. Poor reproducibility was assessed for ICCs below 0.40, fair reproducibility for ICCs between 0.40 and 0.59, good reproducibility for ICCs between 0.60 and 0.75, and excellent reproducibility for ICCs between 0.75 and 1.00 (I-Kuei Lin, 1989).

Bland and Altman, has described an alternative method to look for systematic differences between inter- and intra-examiner readings of methods based on continuous data and to calculate 95% limits of agreement. The upper and lower limits of agreement, between which 95% of repeated readings were expected to lie, were then represented by DIFF ± $2S.D._{DIFF.}$ The range represents the interval between the upper limit of agreement (DIFF + $2S.D._{DIFF}$) and the lower one (DIFF - $2S.D._{DIFF}$) (Bland and Altman, 1986; Bland and Altman, 1990)

1.5 Summary of Literature Review and Overall Aim:

In summary, dental caries continues to be one of the most prevalent disease and a significant burden for the health systems. In recent years evidence has shown the limitation of relying on a restorative approach to manage dental caries. The current biological understanding of the caries process has led to develop new philosophies based on early detection, preventive management and preservation of tooth structure. With the decline in caries prevalence over the last few decades, the pattern of caries has changed

and is characterised by a low prevalence and slow progression. This has made caries detection more difficult, especially early non-cavitated lesions on the occlusal surfaces of permanent teeth. This has increased the need to develop not only accurate diagnostic tools, but tools with a high degree of reliability to measure lesion severity over time to monitor whether caries preventive measures have been successful in bring about lesion arrest or even remineralisation.

Although clinical detection methods are highly specific, the low sensitivity achieved, particularly for non-cavitated occlusal surfaces *in vivo*, means that the use of diagnostic aids with superior performance is indicated and that new methods for caries detection are required. As such, other caries detection devices have been developed recently to provide objective measurements. Examples of this are the DIAGNOdent, the more recently developed DIAGNOdent Pen, and a newer device called the CarieScan PRO, which relies on Electrical Impedance Spectroscopy.

Over the last three decades, there has been a revolution in the medical CT technology in parallel with the invention of compact machines such as cone beam CT which has the potential to provide a reasonable results for caries detection and Micro-CT which can be used in laboratory studies for quantification of mineral loss in carious lesions and validation of caries detection methods. These methods have the potential to be valuable tools in dentistry by producing three dimensional images of the teeth and related structures. However, further studies are required to investigate the usefulness of such methods in caries research.

Therefore, in the first part of this thesis, the main aim is to assess the reproducibility of conventional (ICDAS and Bitewing) and novel (FOTI, CBCT, DIAGNOdent Pen and

CarieScan PRO) detection methods to detect naturally-occurring occlusal and proximal caries in extracted permanent human teeth.

The second part of this thesis will aim to compare between histology and Micro-CT for detection of naturally-occurring occlusal and proximal caries lesions in extracted permanent human teeth.

The third part of the thesis will aim to compare between histology and Micro-CT for validation of conventional and novel caries detection methods for naturally-occurring occlusal and proximal lesions in extracted permanent human teeth.

The last part of this thesis will aim to develop quantitative analysis of Micro-CT scans performed on naturally-occurring occlusal and proximal caries lesions in extracted permanent human teeth.

CHAPTER TWO

Reproducibility of Conventional and Novel Detection Methods for Occlusal and Proximal Caries Detection on Permanent Teeth-An *In vitro* Study

2.1 Introduction

With the decline in caries prevalence over the last four decades, primarily as a result of topical fluoride and improved oral health awareness in many developed countries (Marthaler, 2004; Pitts and Evans, 1997), detection has become more difficult, especially on the occlusal surfaces of permanent teeth (Kidd *et al.*, 1993). In its early stages, carious lesions can be treated preventively where the lesion can be arrested or even remineralised. If such a preventive approach is to be adopted, early caries detection and monitoring is required to determine the severity and activity of the lesion over time (Zandoná and Zero, 2006). This has increased the need to develop not only accurate diagnostic tools, but tools with a high degree of reproducibility to measure consistently results over repeated tests of the same site under identical conditions (Pine and Ten Bosch, 1996; Pretty, 2006). Currently in clinical practice caries detection is carried out primarily with a visual examination supplemented by bitewing radiographs (BW); whilst the latter has proved to be a valuable tool in the detection of proximal caries (Gordan *et al.*, 2011; Kidd and Pitts, 1990) neither method gives an objective reading for comparison over repeated observations.

Other caries detection methods which rely on the subjective interpretation of the visual and radiographic appearances of teeth include Fiberoptic Transillumination (FOTI), Panoramic Radiographs (DPT) and Cone Beam-CT which has the potential to be a valuable tool in dentistry by producing three dimensional images of the teeth and related structures (Haiter-Neto *et al.*, 2008; Côrtes *et al.*, 2000). In an attempt to eliminate some of the subjectivity associated with these detection techniques, other diagnostic devices have been developed to provide objective measurements, for example the DIAGNOdent, and more recently the DIAGNOdent Pen, and electrical conductance methods namely

the Electronic Caries Meter (ECM) and the newer device, the CarieScan PRO, which relies on Electrical Impedance Spectroscopy.

This *in vitro* study therefore aims to determine the reproducibility of conventional visual (ICDAS II) and BW radiographic examination together with novel detection methods (Fiberoptic Transillumination, Cone Beam-CT, DIAGNOdent Pen and CarieScan PRO) for the detection of occlusal and proximal caries in permanent premolar and molar teeth.

2.2 Materials and Methods

2.2.1 Ethical Approval:

Ethical approval for this entire study was obtained and registered by R&D office at Tayside and management approval granted to proceed under REC Ref: 11/AL/0317 and Tayside Ref: 2011DE03 and carried out in accordance with current ethical guidelines (Appendix 2.1).

2.2.2 Sample Selection and Characterization:

Sample Selection Criteria

Two hundred, non-cavitated, extracted permanent posterior teeth (n= 100 premolar teeth and n=100 molar teeth) were selected from a collection of teeth obtained anonymously in Dundee Dental Hospital and School prior to September 2006 and stored in plastic containers under water. The teeth selected were those with representative anatomy of the intended teeth, they were to be assigned to in artificial jaws.

The inclusion criteria for the selected teeth were:

- Sound or non cavitated carious proximal and occlusal surfaces.
- Unrestored teeth.

- No buccal or lingual carious lesions.
- No enamel hypoplasia / hypomineralization defects such as fluorosis.
- No occlusal fissure sealants or extensive fissure staining.

In order to select the 200 teeth, a preliminary visual inspection of approximately 400 teeth was carried out separately by three examiners, recording discreet and reproducibly located investigation sites on the occlusal surface (recorded on black and white photographs) and the worst affected site (if carious) proximally, according to the ICDAS criteria. The three examiners were qualified dentists with at least 13 years' experience and a special interest in restorative dentistry and cariology. One examiner had a particular interest in caries detection and a wealth of experience in ICDAS and radiographic detection of caries. This examiner trained the other two in this respect. Radiographs were also taken of each individual tooth using the correct anatomical orientation and these were also scored (proximal and occlusal surfaces) according to a radiographic depth classification system blindly by the three examiners. Consensus decisions in relation to the ICDAS and radiographic scores for each investigation site and each tooth surface (occlusal and proximal) were used to select a sample of teeth that had a range of sound tooth surfaces and non-cavitated carious lesions of varying appearance and depth to represent a sample with low caries prevalence (for details see Appendix 2.2).

Following this preliminary inspection, the total number of occlusal investigation sites selected in the 200 teeth was 244 (104 sites on the premolar and 140 sites on the molar teeth). All 400 proximal surfaces were also included for examination. The pre-selected investigation sites on the occlusal surface and the worst affected site (if present) on mesial and distal surfaces were marked with circles on black and white photographic prints which were taken at 6x magnification (Leica DC500 CCD camera, Leica Microsystems

Digital Imaging, UK). The letters M and B denoting the mesial and buccal sides of each tooth were marked on the prints of occlusal surface to facilitate relocation of the investigation sites and the orientation of the tooth whilst inserted in phantom heads (Figure 2.1).

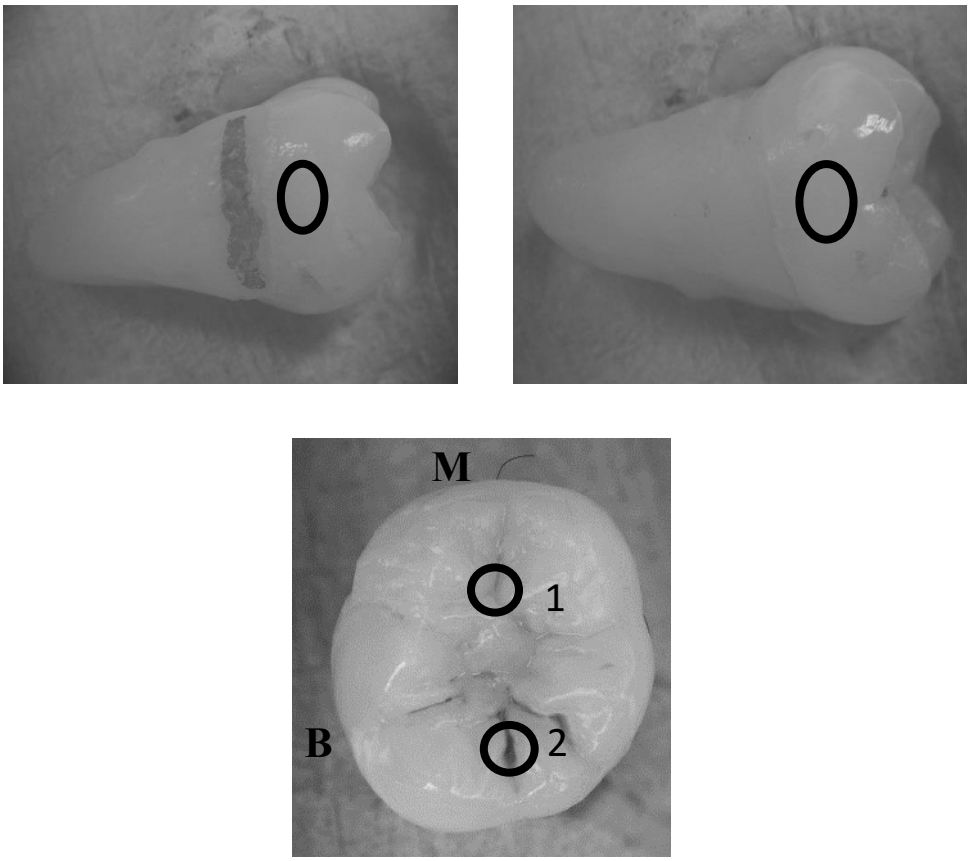


Figure 2.1 Black and white photograph showing the mesial surface (pen mark top left), distal surface top right and the occlusal investigation sites (Bottom Letter M and B are placed to facilitate the orientation of the tooth within the arch upon examination).

Sample Preparation

The selected teeth were cleaned with a tooth brush and ultrasonic scaler to remove any debris, calculus and any remaining soft tissues. Each tooth was initially stored in an individually coded plastic bottle with phosphate buffered saline to which 0.1% thymol crystals were added to prevent bacterial growth and dehydration of the teeth.

All selected teeth were identified to tooth type (premolar/molar; maxillary/mandibular; left/right), to enable anatomically correct dental arches to be created with 2 premolar and 2 molar teeth in each quadrant. To enable orientation of each tooth throughout the study a groove was cut on the mesial aspect of the root and highlighted in indelible red pen. The buccal root surface was marked with a black indelible pen.

2.3 The Main Study

Overview

Colour photographs of the occlusal and proximal surfaces of each tooth were taken and placed into a PowerPoint presentation in order to keep a baseline record of the appearance of the teeth. The occlusal investigation sites were marked with a ring to ensure all examiners examined the same sites; where multiple occlusal investigation sites were chosen the apparently worst affected site was always included. If carious, the worst affected site proximally was also marked with a ring.

To avoid any impact of investing the teeth in die stone, the AC Impedance Spectroscopy (CarieScan PRO) readings were carried out first on the occlusal surfaces only. The teeth were then be set up in arches and placed in phantom heads for the examination of the selected investigation sites on the occlusal and proximal surfaces using the ICDAS visual classification system, a Laser fluorescence device (DIAGNOdent Pen) and FOTI.

Bitewing radiographs of teeth were taken with the maxillary and mandibular arches mounted on an articulator. Panoramic radiographs and Cone beam CT scans were taken with the maxillary and mandibular arches held together with a rubber band to ensure proper occlusal relationship and to avoid any movement.

Fifty percent of the occlusal sites and proximal surfaces were randomly selected for re-examination and rescoring using each examination technique after a period of 1-2 weeks to determine intra-examiner reproducibility.

2.3.1 Selection of Examiners:

Eleven examiners (eight male and three female) were recruited to take part in this study. They were all qualified experienced dentists with at least five years post graduate experience; four were Postgraduate students with an interest in Restorative Dentistry, four were Consultants in Restorative Dentistry and three were Lecturers or Specialty dentists in a restorative or paediatric discipline.

Due to examiners' commitments and the time consuming nature of some of the examinations only five examiners performed the DIAGNOdent Pen examinations on occlusal surfaces and three on proximal surfaces.

2.3.2 AC Impedance Spectroscopy (ACIST) Examination:

A training session was provided for all examiners in the use of the CarieScan PRO^{TM} (CarieScan Ltd, Scotland, UK), this involved the theoretical background (see section 1.2.6) and practical aspects of its use. Prior to making recordings with the CarieScan PRO the teeth were equilibrated for approximately three to four hours inside a sealed glove box under constant conditions namely relative humidity 50-70% and temperature 30-35 °C to closely approximate the intra-oral conditions (Yoshida, 1983; Plasmans *et al.*, 1994). These conditions were confirmed throughout the CarieScan PRO measurement sessions with a digital thermo-hygrometer (TFA Dostmann GmbH &Co. KG, Deutschland) (see Figure 2.2).

Calibration of the CarieScan PRO was carried out according to the manufacturer's recommendations prior to all sessions and during longer sessions. This consisted of two basic operations: testing the electronic system in the device (against a test model of several known impedances) and the electrical flow through the cable and sensor system.

To take the CarieScan PRO reading, the root/s of the teeth were placed into a putty (Blu-Tack®, Bostic Co., USA) held inside a plastic mould. The roots and the putty were completely surrounded in a conductive gel (KY jelly, Johnston & Johnston Co. UK Ltd) into which the oral reference electrode was inserted so completing the circuit (Mickel *et al.*, 2006). Each tooth was examined by each examiner who was blind to other examiners' results.

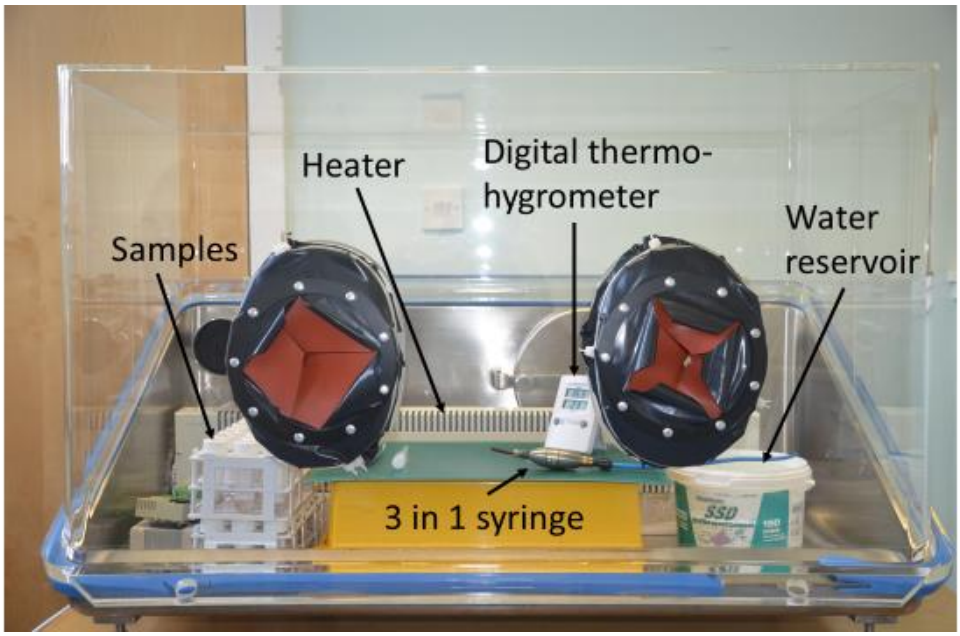
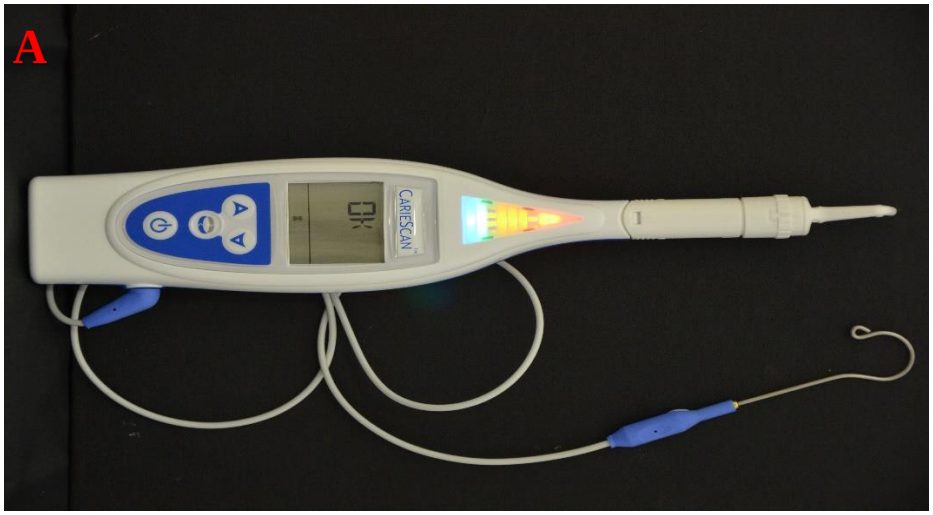


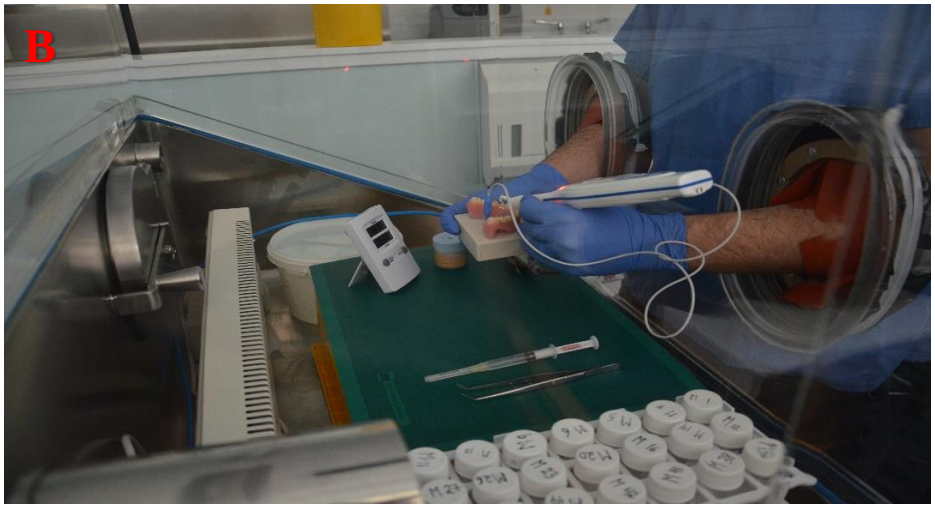
Figure 2.2 Sealed glove box maintained at 50-70% humidity at 30-35 °C used CarieScan PRO reading.

Before examination, the surface under investigation was air dried for 5 seconds using a 3-in-1 syringe to prevent conductance in any remaining surface saline and hence reducing the risk of false positive results. To take the CarieScan PRO reading, the tufted metal wire

sensor was placed at the investigation site/s on the occlusal surface using the black and white Power Point printout (images of the teeth with the investigation sites marked) of the tooth in question to ensure accurate and reproducible positioning of the sensor by each examiner (Figure 2.1).

For each investigation site the numerical reading and colour light displayed on the CarieScan PRO were recorded (Figure 2.3). The performance of the device was assessed according to cut-off limits suggested by the manufacture for enamel and dentine caries (Table 2.1).





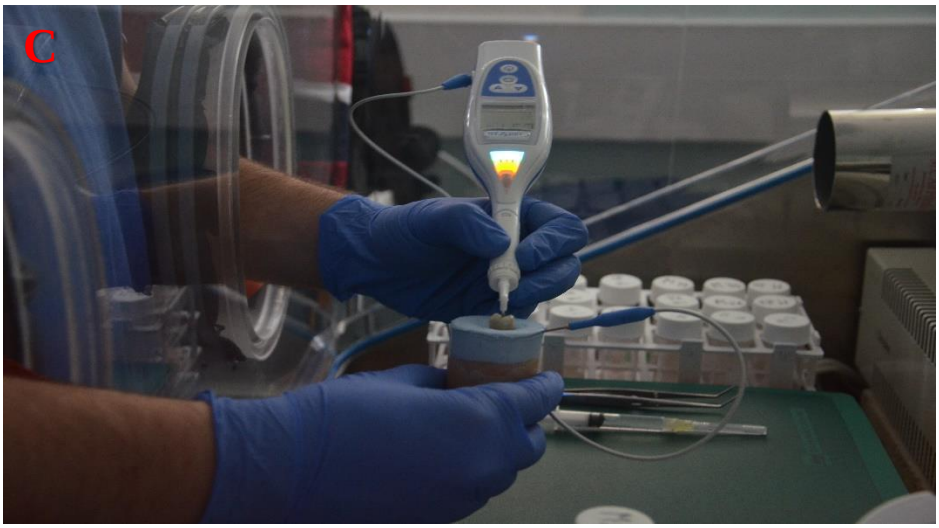


Figure 2.3 Examination of occlusal investigation sites using CarieScan PRO (A) showing numerical Led and light display and reference electrode; (B) CarieScan PRO whilst calibrating against standard calibration model; (C) CarieScan PRO taking the reading where the tooth is inserted in a plastic mould with roots embedded in Blu-Tack putty surrounded in a conductive gel.

Led Display	Site Status	Colour Display
0-20	Sound	
21-30	Outer third enamel caries	
31-50	Middle third enamel caries	
51-90	Inner third enamel caries	
91-99	EDJ and outer half of dentine caries	
100	Inner half of dentine	

Table 2.1 Classification of caries extent according to CarieScan PRO numerical reading and colour displays according to the manufacturer instructions.

Sensors were replaced after scanning 50 teeth, to ensure excessive wear and tear did not affect the readings. Each examiner recorded the readings on the occlusal surface only for the 200 teeth over 3-4 sittings to prevent operator fatigue. At least 1- 2 weeks after all of the teeth had been examined 50% of the investigation sites were randomly selected for re-examination by all examiners to determine intra-examiner reproducibility.

2.3.3 Setting up Teeth in Anatomical Arches:

Following all CarieScan PRO readings the teeth were then set up in anatomical arches in Die trays (Tricodent® die tray system, England).

The Die trays were mounted on an average value articulator at an appropriate vertical spacing to allow positioning of a bitewing film holder between the dental arches.

For this the roots were embedded in pink wax (Dental Modelling Wax Assoc.Dental Products Ltd. Berks, U.K.) up to the expected correct bone levels, ensuring anatomically correct positioning to achieve good contact points and occlusion between opposing arches. Anterior acrylic teeth were added to create a realistic bilateral maxillary-mandibular relationship (Figure 2.4). The positions of teeth were then recorded by taking an addition cured silicone putty impression (Coltène/Whaledent Inc., Switzerland) of the teeth. Once set and removed, the teeth were taken out of the wax and relocated into the correct position in the impression. The roots of the teeth were then invested in Die stone Type IV (Sherapremium, Shera Werkstoff-Technologie, Germany) with added wood chips to resemble cancellous alveolar bone radiographically. When the die stone had set the putty impression was removed, the teeth were re-cleaned and the gingival soft tissues were built up in wax to normal gingival levels. This was again recorded in a second silicone putty impression. The impression was removed, the gingival wax was boiled off and then replaced with Gingifast elastic material (Zhermack Spa, Italy) by reseating the silicone impression. Each pair of jaws representing one head were kept in a separate plastic box filled with phosphate buffered saline to which 0.1% thymol crystals was added to prevent bacterial growth and to avoid teeth dehydrating. The stone arches with embedded teeth were easily removed from and precisely repositioned within the Die trays when used for different types of examination.

A total of 10 complete maxillary and mandibular pairs of jaws were prepared with first and second premolar and molar teeth in each quadrant. A further 3 incomplete pairs of jaws were set up to ensure all selected teeth were used and placed anatomically in the correct position within the arch.

The order of subsequent examination types was randomly selected for each examiner. Similarly, when 50% of teeth were re-examined, the order of examination was also randomly allocated.

Figure 2.4 (A) A pair of jaws with first and second premolar and molar teeth in each quadrant arch and anterior acrylic teeth, (B) an occlusal view of one of the arches (note: upper rigt1st Molar was an example of hypomineralised tooth).

2.3.4 International Caries Detection and Assessment System (ICDAS) Examination:

The examiners initially used the ICDAS e-learning programme to become familiar with the ICDAS visual-tactile examination procedure and criteria (www.icdas.org). First, the examiners completed the e-learning programme released by ICDAS organization through its website, in a 90-min session. Followed, the principle examiner then trained the other examiners in the ICDAS-II classification system in a 2-hour session. This involved a 30 minute lecture on the ICDAS-II system and the details of each score were discussed using a series of images of the occlusal and proximal surfaces of teeth. Following this, a series of approximately 20 projected, magnified images of the occlusal and proximal surfaces

of teeth with a range of appearances were discussed and a consensus ICDAS score made. This was followed by examination of approximately 20 hand-held extracted teeth that were not included in the main study, representing all ICDAS-II scores. These teeth were initially examined independently, followed by discussion and a consensus score given.

code	Extension and appearance of carious lesion
0	**Sound tooth structure** *(no evidence of caries or change in enamel translucency after air drying for 5 seconds)*
1	**First visual change on dry enamel** *(seen only after air drying for 5 seconds or restricted to the pit and fissure seen wet or dry)*
2	**Distinct visual change on moist enamel** *(Brown or white spot opacity/lesion and wider than the natural fissure or fossa seen on a wet surface)*
3	**Localized enamel breakdown with no visible dentine or underlying shadow** *(when dry there is carious loss of enamel but no visible dentine)*
4	**Underlying shadow with or without localized enamel breakdown** *(More noticeable when surface is wet, scored 0 if caries started from adjacent surface unless there are other signs of caries)*

Table 2.2 ICDAS II Classification Criteria for non-cavitated Carious Lesions (Braga *et al.*, 2010a)

For the main part of the study, the ICDAS examinations were carried out with the teeth in the dental arches mounted in a phantom head with a rubber shield to simulate the soft tissues of the cheeks and lips. All examinations were carried out in the Clinical Skills Laboratory at Dundee Dental Hospital and School. Each examiner was given a new disposable dental mirror, WHO type periodontal probe (used gently to confirm the presence of surface discontinuity in enamel), a 3-in-1 air syringe and a dental operating light (Halogen Optic Lamp, OSRAM GmbH, Germany). All investigation sites and each proximal surface were initially examined when wet and then dry according to the ICDAS

criteria (Table 2.2). In the event of doubt as to which code to assign the investigation site the examiners were instructed to choose the code representing the less severe status. All teeth were kept wet whilst in the phantom head to avoid dehydration, in addition teeth were examined in a different random order for each examiner to avoid to any risk of a systematic error.

2.3.5 DIAGNOdent Pen (Laser Fluorescence) Examination:

All examiners were trained in the use of the DIAGNOdent Pen prior to the study. All of the occlusal surfaces were examined first by five examiners in 2 to 3 sessions followed by 3 examiners on proximal surfaces in separate sessions. Before each measurement cycle the DIAGNOdent Pen was calibrated using the ceramic standard kit according to the manufacturer's instructions, ensuring no deviation greater than 3 registered from the standard value.

Prior to examination, each tooth was air dried for about five seconds with the 3-in-1 air syringe. The DIAGNOdent pen was then calibrated for each tooth, selecting an obviously sound site to set as a zero value. The cylindrical sapphire tip with truncated cone end was used for calibration and measurements on the occlusal investigation sites. Circular movements were performed at each test site to ensure the maximum reading for that site was obtained.

For the proximal surface the wedge-shaped sapphire tip was used and this was introduced underneath the contact area, making sure that the bevelled end was facing away from the surface to be examined, first from the buccal side and then from the lingual side. The highest value for each proximal surface was recorded for each examiner (Figure 2.5).

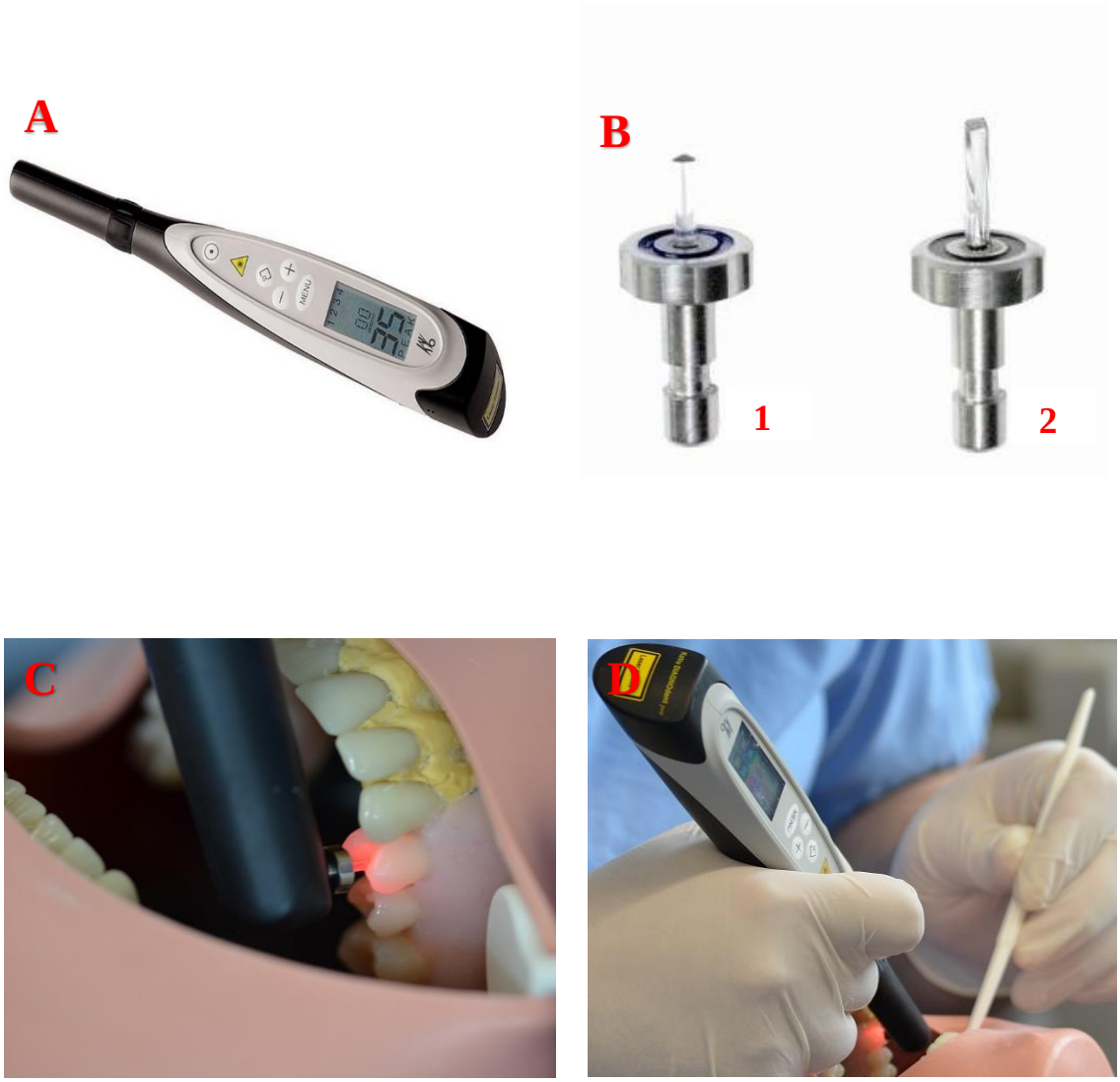


Figure 2.4 (A) Kavo DIAGNOdent Pen (2190); (B) occlusal (1) and (2) proximal sapphire tips; (C) occlusal sapphire tip placed directly on the investigation site; (D) numerical display with the reading using Kavo DIAGNOdent Pen (2190)

Between one and two weeks later, the same randomly selected teeth (50%) were re-examined by each examiner who was blind to the original readings in order to assess intra-examiner reproducibility. The performance of the device was assessed according to cut-off limits suggested by Lussi and co-workers (Lussi and Hellwig, 2006; Lussi *et al.*, 2006a) (Table 2.3)

Score		EXTENSION OF CARIES
Occlusal	Proximal	
0-6	0-6	Sound tooth surface
6.1-13	6.1-9	Enamel carious lesions
13.1-17	9.1-15	Initial dentine carious lesions
> 17	>15	Advanced dentine carious lesions

Table 2.3 Recommended cut-off points for DIAGNOdent Pen on occlusal and proximal surfaces ((Lussi and Hellwig, 2006; Lussi *et al.*, 2006a).

2.3.6 Fiberoptic Transilluminator (FOTI) Examination:

For the FOTI examinations a Microlux™ Transilluminator (AdDent,Inc. USA) was used. The unit comes with either a 2 or 3mm diameter autoclavable light guide; The 2 mm tip was used in this study and all FOTI examinations were carried out with the operating light turned off. (Figure 2.6).

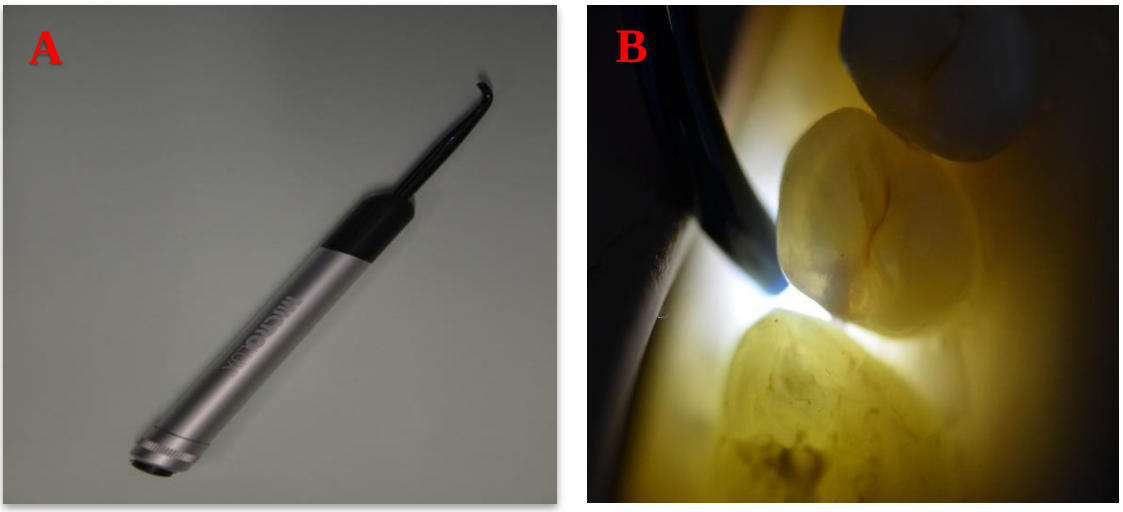


Figure 2.6 (A) MICROLUX™ TRANSILLUMINATOR (AdDent,Inc. USA) showing (B) proximal lesion distal to the 2nd premolar and occlusal lesion on the 1st molar tooth using a 2mm tip.

During the FOTI examinations the examiners were instructed to position the tip and light source buccally and lingually at the cervical margin of the tooth for occlusal caries detection and beneath the contact point for approximal caries detection, and in both cases

directing the light occlusally. The teeth were initially examined wet and then dry in an attempt to enhance discrimination between sound surfaces and enamel lesions. The dark shadow created by any carious lesion was recorded according to the criteria in Table 2.4, which was modified from that described by Côrtes *et al* (Côrtes *et al.*, 2000).

code	FOTI Examination Criteria
0	No shadow or shadow of stain only
1	Thin grey shadow into enamel at the base of the fissure or beneath the marginal ridge affecting the outer half of enamel
2	Wide grey shadow into enamel at the base of the fissure or beneath the marginal ridge (deep) affecting the inner half of the enamel up to the EDJ
3	Orange/brown shadow into dentine <2mm in bucco-lingual direction when seen occlusally or <2mm in mesio-distal direction when seen proximally anticipating to affect the outer third of dentine
4	Orange/brown shadow into dentine >2mm in bucco-lingual direction when seen occlusally or >2mm mesio-distal direction when seen proximally anticipating to affect the middle third of dentine
5	Extensive shadow in dentine when seen occlusally or proximally anticipating to affect the pulpal third of dentine and/or light is blocked

Table 2.4 FOTI Examination criteria for enamel and dentine caries on occlusal and proximal surfaces (modified from criteria described by Côrtes *et al*, 2000).

2.3.7 Radiographic Examinations:

Three radiographic modalities were investigated in this study, namely digital bitewing, standard Dental Panoramic Tomogram (DPT) and Cone beam-CT (either in set panoramic mode or mesio-distal tomograms). Each examiner viewed each type of radiograph and classified the occlusal and proximal surfaces according to a modified version of the criteria developed by Ricketts *et al.,* (Ricketts *et al.*, 2002) and presented in Table 2.5. For all radiographic modalities no soft tissue equivalent material was used. All images were exported and saved as TIFF files. The images were displayed on a 17-inch monitor (Dell Computer Corporation, Austin, TX, USA) at a resolution of 1280 x

1024 pixels under dimmed ambient lighting conditions. All of the examiners viewed all of the radiographic images under standardised conditions, in the same room and on the same computer screen. Each examiner had the option to adjust the brightness and the contrast of the images in order to optimize the quality of the images.

code	Extension of carious lesion
0	sound
1	Caries (radiolucency) in the outer half of enamel
2	Caries (radiolucency) in the inner half enamel
3	Caries (radiolucency) in the outer third of dentine
4	Caries (radiolucency) in the middle third of dentine
5	Caries (radiolucency) in the inner third of dentine

Table 2.5 Radiographic Classification Criteria of carious lesions.

- **Digital Bitewing Radiograph**

To produce the digital bitewing radiographs, each pair of arches were mounted on an articulator which was placed on a flat bench surface (Figure 2.7). A ring bitewing film holder (DENTSPLY Rinn's XCP bitewing film holder, USA) was used to position the digital receptor (Phosphor Plate size 2) and x-ray set collimator. All images were taken using a wall-mounted X-ray unit (Heliodent DS, Sirona Dental Systems GmbH, Germany) operated at 60 kVp and 10 mA, with a 0.40 second exposure time, using a 34.5 cm source-to-image receptor collimator. The phosphor plates were loaded into cassettes which were read out using Vista Scan Perio Plus scanner (Durr Dental AG, Germany) to create 16 bit images; all images were processed by SIDEXIS Next Generation 1.53 (Sirona, Germany) imaging software on a PC unit (Microsoft windows XP).

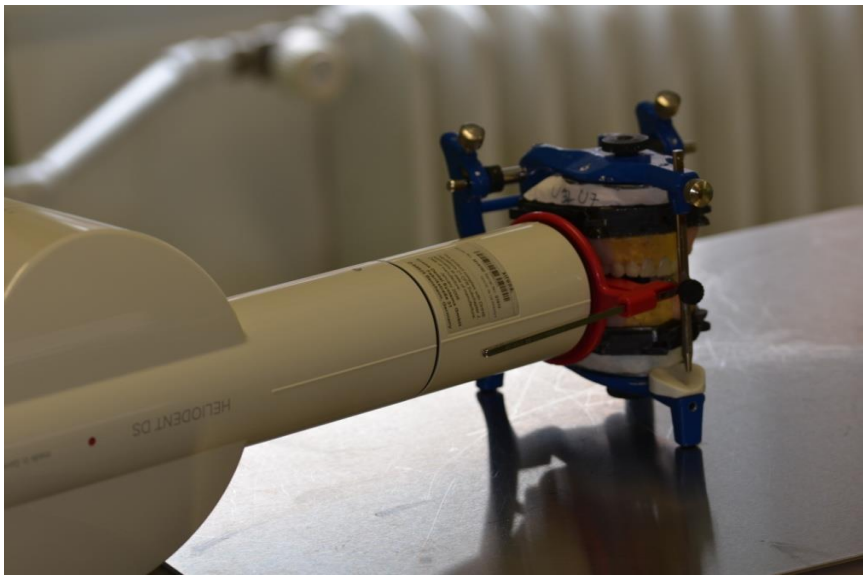


Figure 2.7 Digital Bitewing Radiograph technique for a pair of arches mounted on an articulator.

- **Digital Dental Panoramic Tomograms (DPT)**

Standard DPT images were obtained using a 2 D Digital Panoramic x-ray machine (OrthophoXG5, Sirona, USA) and operated according to the manufacturer settings (see Figure 2.8).

Figure 2.8 Digital OrthophoXG5 Panoramic x-ray machine.

For the DPT images, the arches were mounted in simple plastic hinge articulators with the paired arches placed on the chin positioning platform and oriented using the midline

plane adjusted between the upper central incisors, the Frankfurt horizontal plane and the canine indication plane adjusted as for patients. The machine was set at 64 KV, 8mA, and at exposure time 14.1 seconds with CCD sensor of 27µm pixel size to capture images. The DPT machine was used at programme one (Standard panoramic radiograph with orthoradial projection) to create 16 bit images; all images were processed by SIDEXIS Next Generation 1.53 (Sirona, Germany) imaging software on a PC unit (Microsoft windows XP).

- **Cone Beam Computed Tomography (CBCT)**

To produce the Cone Beam CT images the paired upper and lower arches with the selected teeth were scanned under standardized conditions using i-CAT®Imaging Cone Beam next generation CT system (Imaging Sciences International, USA) and operated according to the manufacturer settings (Figure 2.9).

The heads were placed on a positioning platform and oriented in such a way that the coronal positioning line (light guide) was aligned between lower right second premolar and first molar teeth, the sagittal positioning line (light guide) was aligned between upper central incisors and the horizontal positioning line (light guide) was parallel to the occlusal plane. The machine was operated at 120 KV, 37.07 mAs and at an acquisition time of 26.9 seconds. All scans were acquired at 0.125 mm resolution with Field Of View (FOV) of 4 cm by 16 cm in diameter. The images were reconstructed using i-CAT vision (Imaging Sciences International, USA) software on a PC unit (Microsoft windows XP). This allowed serial tomographic images of the teeth to be viewed in any plane including sagittal, cross-sectional (horizontal), coronal views and panoramic views. For each pair of arches an ideal panoramic 2D image of 0.15 mm thickness was created from the 3D

data by a Consultant Dentomaxillofacial Radiologist by manually drawing a curve in the mid-coronal area of each of the teeth in the lower arch. The image created was saved in a TIFF format file.

Each examiner viewed the serial Cone Beam CT images of each tooth in the sagittal (mesio-distal) plane; the same plane that the teeth were ultimately planned to be imaged using Micro-CT and to be sectioned for histological validation purposes. This was done by each examiner moving the cursor through the image slices from the buccal to lingual side of the tooth and vice versa and recording the deepest depth score of the lesion, if present, on the occlusal and proximal surfaces.

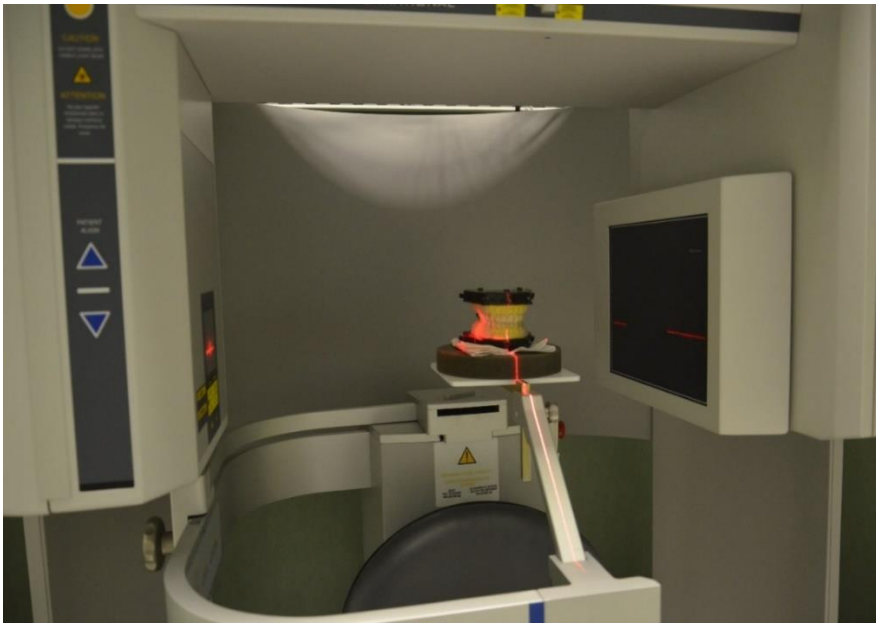


Figure 2.9 i-CAT®Imaging Cone Beam next generation CT system, showing the alignment light guides.

2.3.8 Statistical Analysis:

Descriptive statistics (frequencies) were used to summarize the distribution of scores given by each examination/classification technique for each examiner, using SPSS

software V 21(PASW statistics, SPSS Inc., Chicago, USA) and Excel 2013 (Microsoft Corporation, USA).

For all categorical data (using the entire classification scale for ICDAS, FOTI and all the Radiographic scores), Cohen's unweighted kappa was applied to assess inter- and intra-examiner reproducibility using SPSS software. The weighed kappa statistic (Landis and Koch, 1977) was also used to evaluate inter- and intra-examiner agreement. All weighted kappa analyses were carried out using MedCalc V 13.1.2.0 statistical software (MedCalc Software bvba, Belgium), and the level of significance was set at $P < 0.05$. The continuous data from the DIAGNOdent Pen and CarieScan PRO were converted into categorical data according to Table 2.6 and Table 2.7. These tables represent a version modified from the cut-off points presented in Table 2.1 and 2.3 in order to establish comparable unweighted and weighted kappa values. The modification permits caries within enamel to be categorised as affecting either the outer or the inner half of enamel.

For those detection techniques that recorded continuous data, (DIAGNOdent Pen and CarieScan PRO), the intra-class correlation coefficient (ICC) was used to assess the inter- and intra-examiner reproducibility.

Limits of agreement were also used to look for systematic differences for inter- and intra-examiner reproducibility using DIAGNOdent Pen and CarieScan PRO readings (Bland and Altman, 1986; Bland and Altman, 1990).

The categorical data for all techniques were also dichotomised to establish the inter- and intra-examiner reproducibility, using mean Cohen's kappa values at the D_1 diagnostic threshold (any lesion in enamel or dentine classed as caries) and at the D_3 diagnostic threshold (only lesions in dentine classed as caries).

Score	Extension of Caries	Occlusal Reading	Proximal Reading
0	Sound	0-6	0-6
1	Outer half enamel caries	6.1-9.5	6.1-7.5
2	Inner half enamel caries	9.6-13	7.6-9
3	Outer half of dentine	13.1-17	9.1-15
4	Inner half of dentine	>17	>15

Table 2.6 Categorical Classification criteria of caries extent and DIAGNOdent Pen readings (modified version of Lussi and co-workers (Lussi *et al.*, 2006a; Lussi and Hellwig, 2006)).

Score	Extension of Caries	Led Display
0	Sound	0-20
1	Outer half enamel caries	21-50
2	Inner half enamel caries	51-90
3	Outer half of dentine	91-99
4	Inner half of dentine	100

Table 2.7 Categorical Classification criteria of caries extent and CarieScan PRO readings (modified version of the manufacturer instructions).

2.4 Results

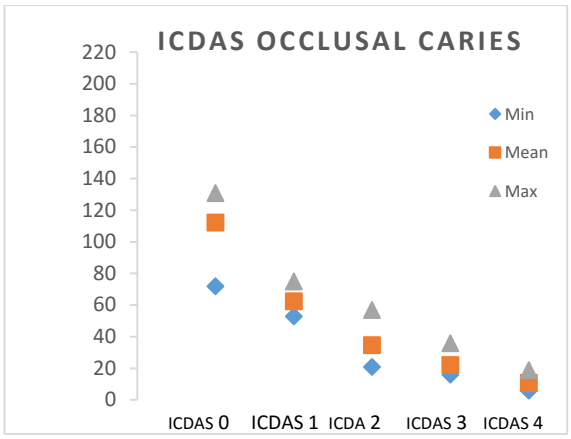
2.4.1 Inter- and Intra-examiner Reproducibility for Occlusal Caries:

The frequency distribution of ICDAS and FOTI scores for each examiner (mean, minimum and maximum) for all 244 specific occlusal investigation sites is presented in Figure 2.10; and for digital bitewing radiographs, Cone Beam-CT, Panoramic view (CBCT) and DPT scores for all 200 occlusal surfaces is presented in Figure 2.11.

The Cohen's kappa values and weighted kappa (mean and SD) values for inter- and intra-examiner reproducibility for ICDAS, FOTI, Digital Bitewing Radiographs, Cone Beam-

CT, Panoramic view (CBCT) and DPT occlusal caries examinations for all examiners are presented in Table 2.8. Corresponding values for CarieScan PRO and DIAGNOdent Pen readings when converted into categorical data can also be seen in Table 2.8. This shows that for categorical data inter-examiner reproducibility for CarieScan PRO and DPT were inferior to the other examination techniques, whereas the DIAGNOdent Pen was superior showing substantial agreement. For all other examination techniques the inter-examiner reproducibility was of moderate agreement. The intra-examiner reproducibility for CarieScan PRO was inferior to the other examination techniques, whereas for all other examination techniques the intra-examiner reproducibility was of moderate to substantial agreement.

Concerning the DPT radiographs proximal overlap was a major problem affecting both the ability to interpret the proximal surface and also the occlusal surface. For each examiner between 5 and 75 occlusal surfaces were deemed unsuitable to make a decision. Only just over half (n=113; 56.5%) of the occlusal surfaces were scored by all 11 examiners; the problem in relation to proximal caries detection was significantly worse than for occlusal caries detection. The data presented in Table 2.8 for the DPT examination relates only to those surfaces scored by all examiners and by the same examiners on different occasions.



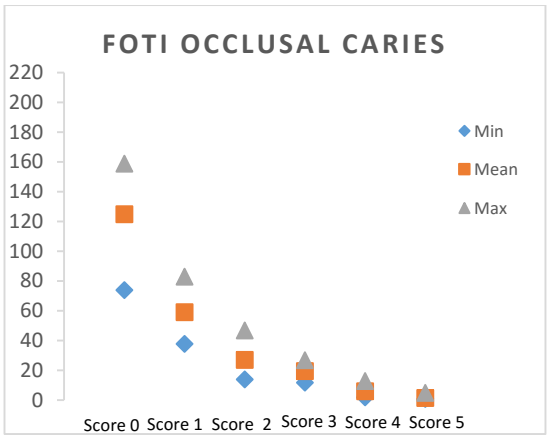
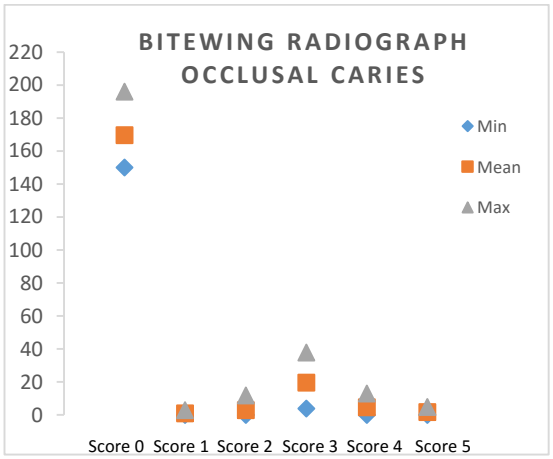
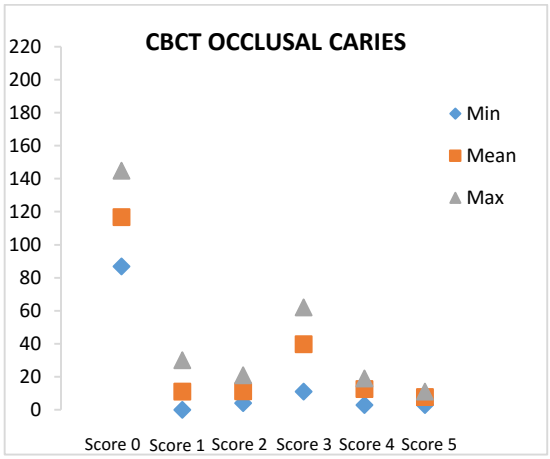
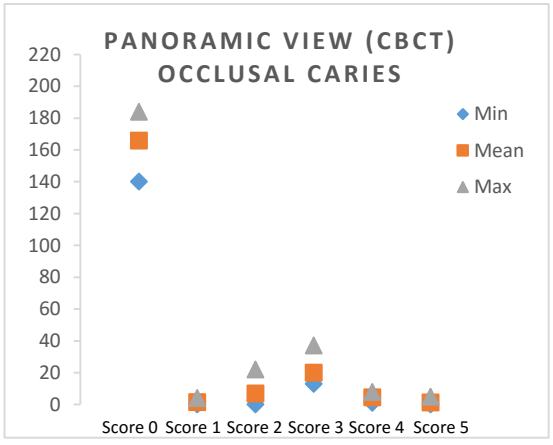


Figure 2.10 Frequency distribution of ICDAS and FOTI scores achieved by all examiners for all 244 occlusal investigation sites







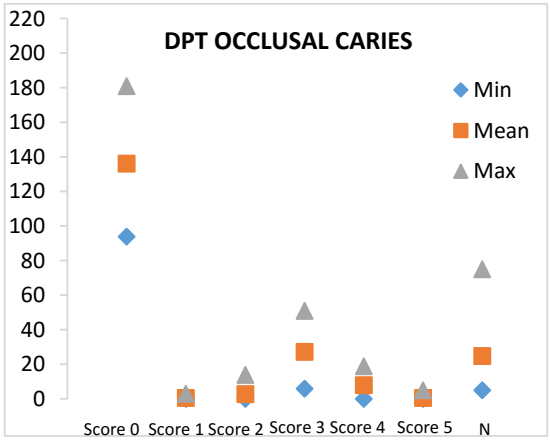


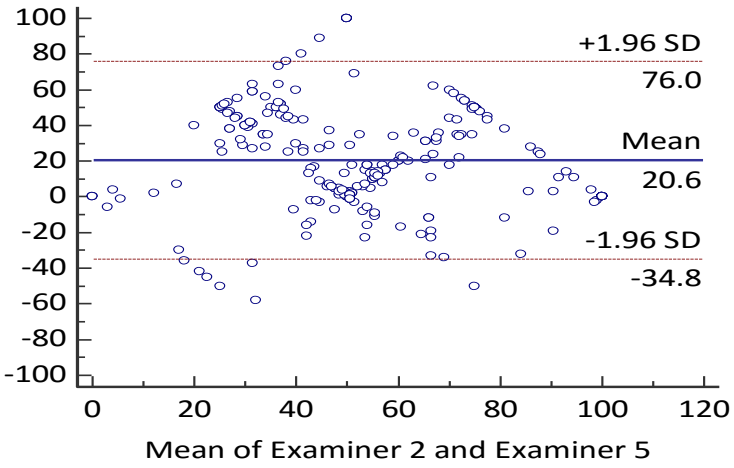
Figure 2.11 Frequency distribution of Digital Bitewing Radiograph, CBCT, Panoramic view (CBCT) and Digital Panoramic Radiograph scores achieved by all examiners for all 200 occlusal surfaces.

		Inter-examiner reproducibility Mean (SD)		Intra-examiner reproducibility Mean (SD)	
	N	Unweighted kappa	Weighted kappa	Unweighted kappa	Weighted kappa
DIAGNOdent Pen	5	0.68 (0.02)	0.71 (0.02)	0.71 (0.02)	0.76 (0.02)
FOTI	11	0.51 (0.07)	0.58 (0.07)	0.59 (0.06)	0.67 (0.06)
ICDAS	11	0.50 (0.12)	0.56 (0.14)	0.61 (0.06)	0.68 (0.06)
CBCT	11	0.46 (0.09)	0.55 (0.10)	0.50 (0.10)	0.58 (0.11)
Panoramic view of CBCT	11	0.39 (0.06)	0.45 (0.07)	0.52 (0.09)	0.58 (0.09)
Bitewing Radiographs	11	0.36 (0.10)	0.40 (0.10)	0.39 (0.12)	0.41 (0.11)
DPT	11	0.31 (0.06)	0.34 (0.06)	0.38 (0.12)	0.42 (0.12)
CarieScan PRO	11	0.29 (0.09)	0.32 (0.07)	0.33 (0.09)	0.39 (0.08)

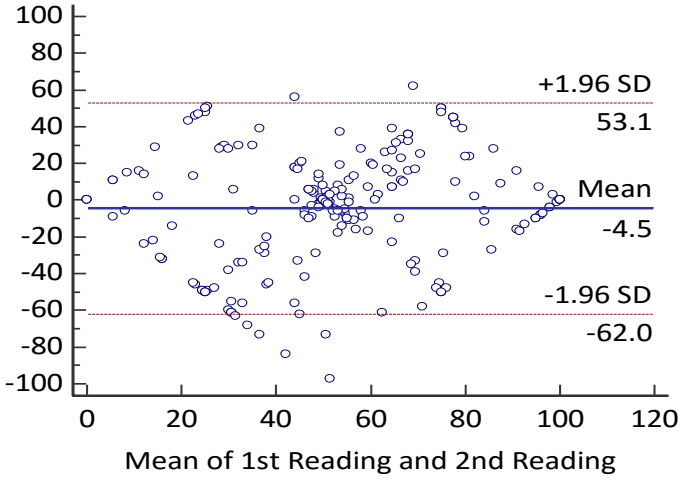
Table 2.8 Summary of Inter- and Intra-examiner Reproducibility for occlusal Caries Detection (unweighted and weighted kappa); N= number of examiners; cell highlighted with green colour indicates substantial agreement, cells highlighted with yellow colour indicate moderate agreement and cells highlighted with red colour indicate fair agreement.

The ICC $_{Mean}$ value (95% CI) for inter-examiner reproducibility using the CarieScan PRO on the 244 occlusal investigation sites was 0.33 (0.24-0.44) while the intra-examiner reproducibility ICC $_{Mean}$ value was 0.36 (0.25-0.48) indicating poor agreement between and within the examiners.

The limits of agreement (mean±1.96 SD) for repeated CarieScan PRO readings can be seen in the example in Figure 2.12 (A) for inter-examiner and (B) for intra-examiner reproducibility. The average mean difference between repeated readings (SD) for inter- and intra-examiner reproducibility were 7.9 (1.3) and -2.6 (3.1) respectively. The average range of the upper and lower limits of agreement for inter- and intra-examiner reproducibility was between 61 and -59, 52 and -53 respectively.



(A)



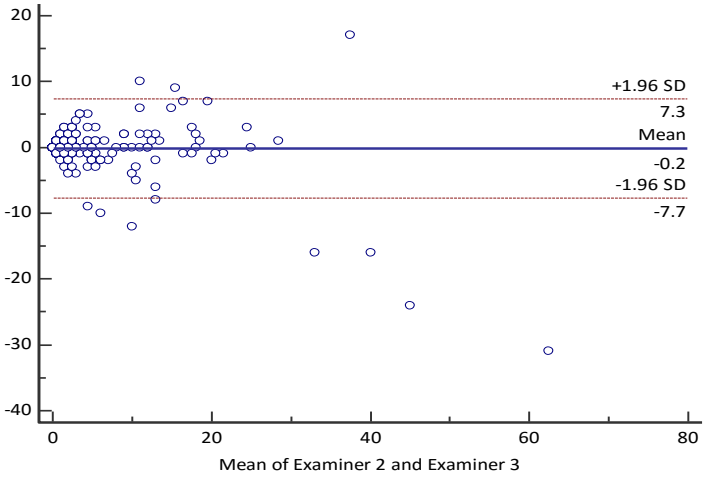
(B)

Figure 2.12 Example of Bland –Altman plot for Inter-examiner (between examiner 2&5) (A) and Intra-examiner (for examiner 2) (B) reproducibility of CarieScan PRO for occlusal caries

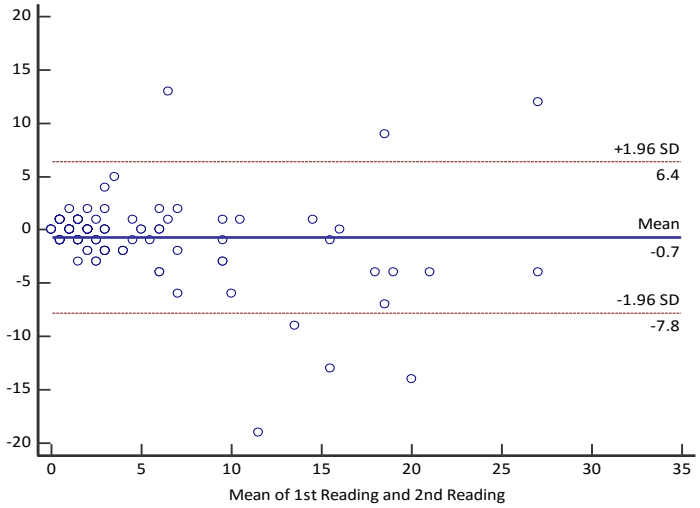
The ICC $_{Mean}$ value (95% CI) for inter-examiner reproducibility using the DIAGNOdent Pen on the 244 occlusal investigation sites was 0.88 (0.86-0.91) while the intra-examiner reproducibility ICC $_{Mean}$ value was 0.83 (0.78-0.86) indicating excellent agreement between and within the examiners.

The limits of agreement (mean±1.96 SD) for repeated DIAGNOdent Pen readings can be seen in the example in Figure 2.13 (A) for inter-examiner and (B) for intra-examiner reproducibility. The average mean difference between repeated readings (SD) for inter- and intra-examiner reproducibility was 0.1 (0.3) and -0.7(0.2) respectively. The average

range of the upper and lower limits of agreement for inter- and intra-examiner reproducibility was between 7 and -8, and 6 and -8 respectively.



(A)



(B)

Figure 2.13 Example of Bland –Altman plot for Inter-examiner (between examiner 2&3) (A) and Intra-examiner (for examiner 3) (B) reproducibility of DIAGNOdent Pen for occlusal caries.

For each examiner and each examination method the results were converted into dichotomous data, using specific cut-off points for each examination method to obtain kappa values for the D_1 and D_3 diagnostic threshold. The inter-examiner reproducibility for all examination techniques was calculated using Cohen's kappa statistics. A summary of this data is represented in Table 2.9.

			D_1 Diagnostic Threshold	D_3 Diagnostic Threshold
	Cut-off point D_1	Cut-off point D_3	kappa $_{Mean}$ (SD)	kappa $_{Mean}$ (SD)
ICDAS	**0/1**	**1/2**	0.58(0.10)	0.62(0.09)
ICDAS	**-**	**2/3**	-	0.63(0.07)
FOTI	**0/1**	**2/3**	0.52(0.08)	0.63(0.07)
Bitewing Radiographs	**0/1**	**2/3**	0.39(0.08)	0.60(0.10)
CBCT	**0/1**	**2/3**	0.57(0.09)	0.36(0.11)
Panoramic view of CBCT	**0/1**	**2/3**	0.43(0.06)	0.59(0.08)
Cariescan	**20/20.1**	**90/90.1**	0.18(0.09)	0.46 (0.09)
DIAGNOdent Pen	**6/6.1**	**13/13.1**	0.83(0.04)	0.28(0.09)

Table 2.9 Summary of Inter-examiner Reproducibility for occlusal Caries Detection (Mean of Unweighted kappa for all examiner comparisons) at D_1 and D_3 diagnostic threshold.

2.4.2 Inter- and Intra-examiner Reproducibility for Proximal Caries:

The frequency distribution of ICDAS and FOTI scores for each examiner (mean, minimum and maximum) for all 400 proximal surfaces is presented in Figure 2.14; and for digital bitewing radiographs, Cone Beam-CT, Panoramic view (CBCT) and DPT scores for all 400 proximal surfaces is presented in Figure 2.15.

The Cohen's kappa values and weighted kappa (mean and SD) values for inter- and intra-examiner reproducibility for ICDAS, FOTI, Digital Bitewing Radiographs, Cone Beam-CT, Panoramic view (CBCT) and DPT proximal caries examinations for all examiners are presented in Table 2.10. Corresponding values for DIAGNOdent Pen readings when converted into categorical data can also be seen in Table 2.10. This shows that for categorical data inter-examiner reproducibility for DIAGNOdent Pen and Panoramic

view (CBCT) were inferior to the other examination techniques, whereas the ICDAS was superior showing substantial agreement. For all other examination techniques the inter-examiner reproducibility was of moderate agreement. The intra-examiner reproducibility for DIAGNOdent Pen was also inferior to the other examination techniques, whereas for all other examination techniques the intra-examiner reproducibility was of moderate to substantial agreement.

Concerning the DPT radiographs proximal overlap was a major problem affecting the ability to interpret the proximal surfaces. For each examiner between 121and 220 occlusal surfaces were deemed unsuitable to make a decision. Only a third (n=131) of the proximal surfaces were scored by all 11 examiners; the problem in relation to proximal caries detection was significantly worse than occlusal caries detection. The data presented in Table 2.8 for DPT examination relates only to those surfaces scored by all examiners and by the same examiners on different occasions. For this reason, the data collected from the DPT examination will not be included further in this study.

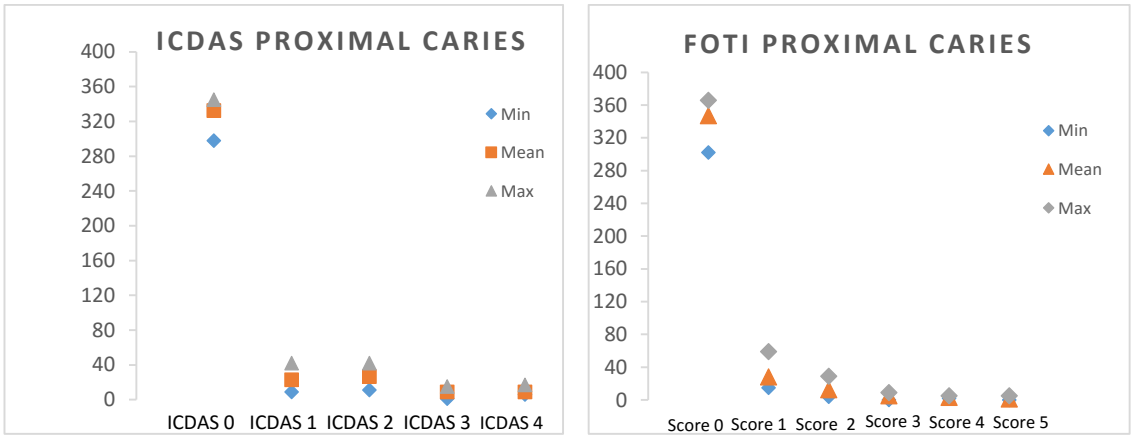


Figure 2.14 Frequency distribution of ICDAS and FOTI scores achieved by all examiners for all 400 proximal surfaces.

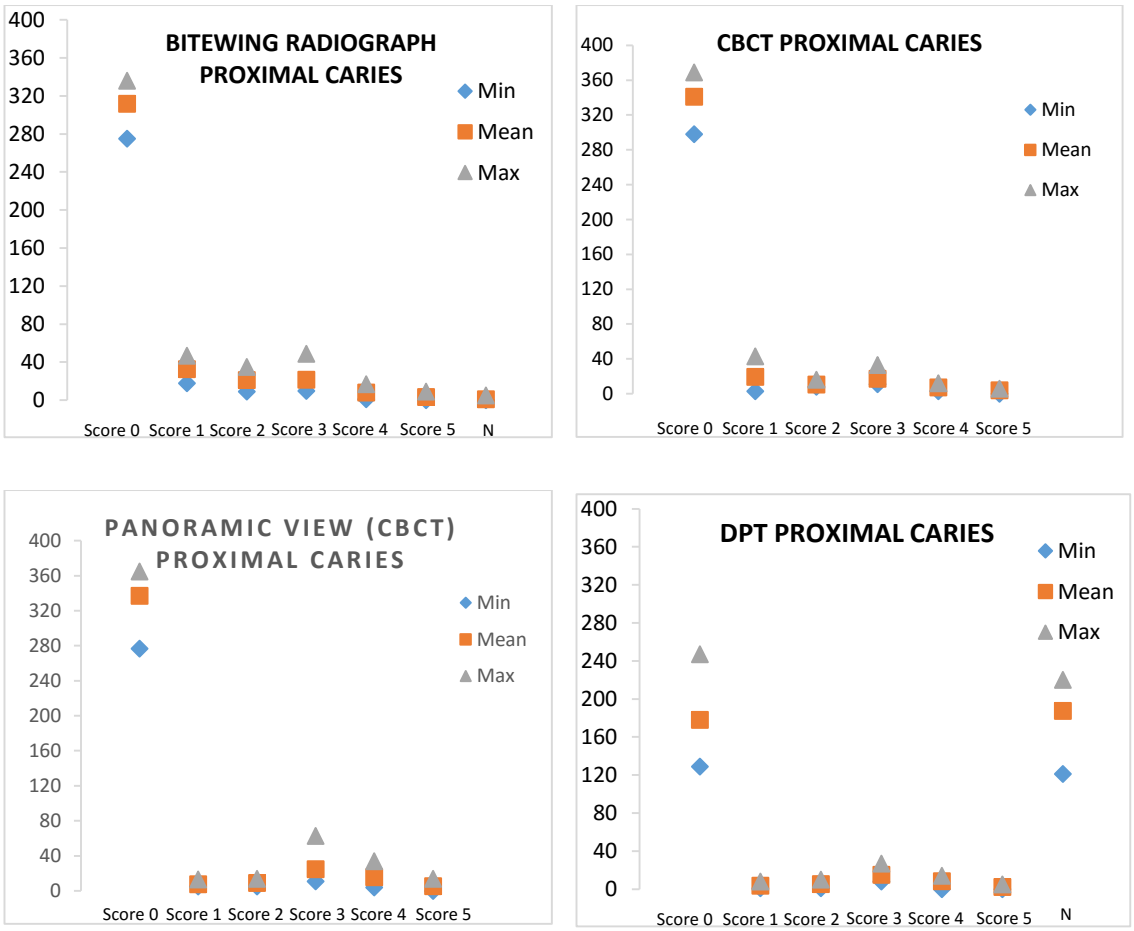


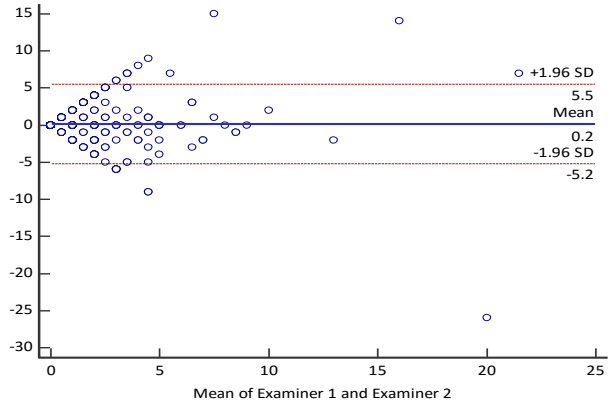
Figure 2.15 Frequency distribution of Digital Bitewing Radiograph, CBCT, Panoramic view (CBCT) and Digital Panoramic Radiograph scores achieved by examiners for all 400 proximal surfaces.

		Inter-examiner reproducibility Mean (SD)		Intra-examiner reproducibility Mean (SD)	
	N	Unweighted kappa	Weighted kappa	Unweighted kappa	Weighted kappa
ICDAS	11	0.53 (0.10)	0.62 (0.07)	0.63 (0.06)	0.70 (0.07)
CBCT	11	0.49 (0.09)	0.59 (0.09)	0.53 (0.10)	0.65 (0.10)
FOTI	11	0.46 (0.07)	0.51 (0.09)	0.53 (0.07)	0.59 (0.09)
Bitewing Radiographs	11	0.36 (0.10)	0.42 (0.07)	0.49 (0.10)	0.55 (0.08)
DPT	11	0.31 (0.06)	0.41 (0.09)	0.48 (0.11)	0.53 (0.12)
DIAGNOdent Pen	3	0.35 (0.02)	0.39 (0.02)	0.29 (0.02)	0.32 (0.02)
Panoramic view of CBCT	11	0.31 (0.06)	0.35 (0.09)	0.50 (0.09)	0.56 (0.09)

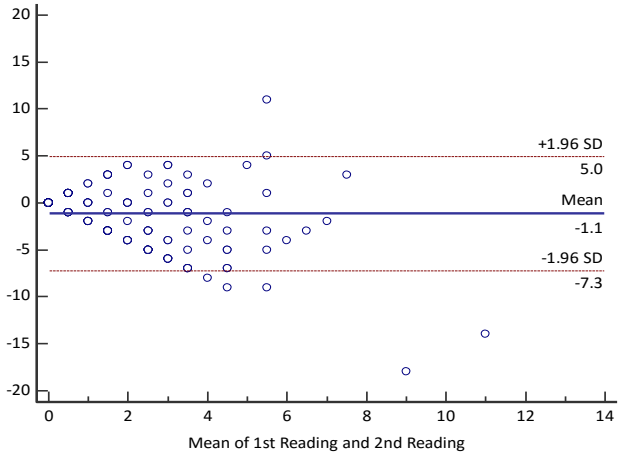
Table 2.10 Summary of Inter- and Intra-examiner Reproducibility for proximal Caries Detection (unweighted and weighted kappa); N= number of examiners; cell highlighted with green colour indicates substantial agreement, cells highlighted with yellow colour indicate moderate agreement and cells highlighted with red colour indicate fair agreement.

The ICC $_{Mean}$ value (95% CI) for inter-examiner reproducibility using the DIAGNOdent Pen on the 400 proximal surfaces was 0.36 (0.3-0.46) while the intra-examiner reproducibility ICC $_{Mean}$ value was 0.24 (0.14-0.36) indicating poor agreement between and within the examiners.

The limits of agreement (mean±1.96 SD) for repeated DIAGNOdent Pen readings can be seen in the example in Figure 2.16 (A) for inter-examiner and (B) for intra-examiner reproducibility. The average mean difference between repeated readings (SD) for inter- and intra-examiner reproducibility was 0.7 (0.2) and -0.2 (0.6) respectively. The average range of the upper and lower limits of agreement for inter- and intra-examiner reproducibility was between 6.5 and -5, and 7 and -6.5 respectively.



(A)



(B)

Figure 2.16 Example of Bland –Altman plot for Inter-examiner (between examiner 1&2) (A) and Intra-examiner (for examiner 3) (B) reproducibility of DIAGNOdent Pen for proximal caries

For each examiner and each examination method the results were converted into dichotomous data, using specific cut-off points for each examination method to obtain kappa values for the D_1 and D_3 diagnostic threshold. The inter-examiner reproducibility for all examination techniques was calculated using Cohen's kappa statistics. A summary of this data is represented in Table 2.11.

			D_1 Diagnostic Threshold	D_3 Diagnostic Threshold
	Cut-off point D_1	Cut-off point D_3	kappa $_{Mean}$ (SD)	kappa $_{Mean}$ (SD)
ICDAS	**0/1**	**1/2**	0.62(0.10)	0.63(0.10)
ICDAS	**-**	**2/3**	-	0.60(0.08)
FOTI	**0/1**	**2/3**	0.49(0.12)	0.60(0.09)
Bitewing Radiographs	**0/1**	**2/3**	0.46(0.09)	0.43(0.09)
CBCT	**0/1**	**2/3**	0.59(0.10)	0.64(0.12)
Panoramic view of CBCT	**0/1**	**2/3**	0.36(0.08)	0.33(0.10)
DIAGNOdent Pen	**6/6.1**	**9/9.1**	0.34(0.14)	0.39(0.10)

Table 2.11 Summary of Inter-examiner Reproducibility for proximal Caries Detection (Mean of Unweighted kappa for all examiner comparisons) at D_1 and D_3 diagnostic threshold.

2.5 Discussion

With the decline in caries prevalence over the last four decades there has been a change in the distribution of carious lesions with occlusal lesions accounting for the majority of lesions in children, adolescents and young adults (Pine and Ten Bosch, 1996; Mejàre *et al.*, 2004). In its early stages, it can be treated preventively where the lesion can be arrested or even remineralised (Pitts, 2004a). As contemporary caries management is preventively led a caries detection method should have an acceptable level of reproducibility in order to achieve effective monitoring of the disease over time (Gimenez *et al.*, 2013c; Ekstrand, 2004). Poor reproducibility could give the impression that a lesion has progressed or arrested (regressed) over time simply because the detection method is inconsistent. Thus the aim of this Chapter was to investigate the intra (within) -examiner and inter- (between) examiner reproducibility.

New human tissue legislation was issued in 2006 that regulates the usage of human tissues, including teeth, for research purposes, the so called "Human Tissue (Scotland) Act 2006" (**www. hta.gov.uk**). The fundamental principle is that any teeth collected after September 2006 requires patient consent and ethical approval for use in research. Teeth collected prior to this date can be used for research pending ethical approval. As such all 200 teeth used in this study were chosen from a selection of extracted teeth collected anonymously and labelled prior to September 2006 and following ethical approval from R&D office at Tayside and management approval granted to proceed under REC Ref: 11/AL/0317 and Tayside Ref: 2011DE03.

A convenience sample size of 200 teeth was included in this study. A power calculation was not possible as histology / Micro-CT which shows the true status of the lesions could not be performed before the detection methods were carried out. As such the true prevalence of disease in the sample could not be known for sure prior to the study commencement. The sample size, prevalence of disease and number of examiners in this study was targeted based upon recommendation from two publications by Bader *et al*., (2002) and Twetman *et al*., (2013). In a systematic review by Bader *et al,* (2002) on the performance of methods for detection of carious lesions it was suggested that studies should include over 150 teeth, with a caries prevalence of less than 20% and more than four examiners to reach a good quality score. A more recent systematic review on adjunctive methods for caries detection by Twetman *et al,* (2013) suggested inclusion criteria for *in vitro* studies should include above 60 teeth with at least three observers.

An ideal laboratory study should be designed under standard conditions without any confounding factors that would affect the outcomes of the detection methods (Pretty, 2006; Zandoná and Zero, 2006). In our study, only non-cavitated teeth were included,

without restorations and any enamel defects that would affect the accuracy of caries detection. The teeth included were mainly premolars extracted for orthodontic reasons, third molars or periodontally compromised teeth. Therefore it was inevitable that some third molars were used in the place of first and second molar teeth and some second premolars were placed in the place of first premolars and vice versa. All teeth were however, eventually mounted in arches in the correct anatomic quadrant, bucco-lingual orientation and to simulate clinical contact points and occlusion. Only third molars with representative anatomy of first and second molars were selected for this study

A preliminary inspection, including visual and radiographic examination, of approximately 400 teeth was carried out to choose a representative sample with varying appearances to reflect a low caries prevalence pattern among populations (Bönecker *et al.*, 2010; Pretty, 2006; Sgan-Cohen *et al.*, 2015; Downer *et al.*, 2005). To achieve this task, three qualified examiners with at least 13 years clinical experience were used. The 200 teeth chosen had a variety of appearances ranging from sound to non-cavitated dentine lesions with underlying shadow. Previous studies have also mentioned that a preliminary inspection had been carried out in order to select an appropriate sample size with representative appearances before carrying out the main study (Kamburoğlu *et al.*, 2010b; Krzyzostaniak *et al.*, 2014). Recent systematic reviews by Gimenez *et al*, (2015a)&(2015b) raised concerns about most *in vitro* studies in that the sample selection and distribution of lesions did not necessarily match the expected prevalence of the target population in mind. As such they concluded that most of the studies had a high risk of sample selection bias. The careful selection of teeth in this study tried to address this issue.

Each occlusal surface had at least one investigation site that would be the worst affected site to allow comparison with surface specific caries detection methods, which are all radiographic modalities. All occlusal investigation sites were marked with circles on white and black photographic images at 6x magnification (Neuhaus *et al.*, 2011). This allowed examiners to easily identify and relocated discrete sites with different appearances. In several *in vitro* studies for investigating the performance of different caries detection methods, multiple occlusal investigation sites have been included to increase the sample size or to differentiate between lesions on the same occlusal surface (Ekstrand *et al.*, 1997; Côrtes *et al.*, 2000; Jablonski-Momeni *et al.*, 2009; Diniz *et al.*, 2011). In teeth with multiple investigation sites it can be argued that each site does not represent independent data as when looking at one site the examiner may be influenced by another. However, in a previous study that looked at visual caries detection on occlusal surfaces with multiple investigation sites, it was shown that the diagnostic performance was unaffected as to whether one or more investigation sites were chosen (Jablonski-Momeni *et al.*, 2012).

All teeth were kept throughout the study in saline to which 0.1% thymol crystals were added to prevent bacterial growth. The use of thymol crystals in solution is a common storage medium for extracted teeth in laboratory studies and is unlikely to affect any of the caries detection methods used in this study (Jallad *et al.*, 2015; Braga *et al.*, 2010c; Côrtes *et al.*, 2003; Gomez *et al.*, 2013b).

Eleven examiners were recruited to take part in this study; all were qualified dentists with various postgraduate experience and with an interest in restorative dentistry. It was not the aim of this study to determine whether there were any variations among examiners' reproducibility due to variations in speciality and clinical experience; there were

insufficient numbers in each group to determine this. However, previous studies have evaluated different groups of examiners, which were graduate dentists, undergraduate dentists and specialists with different levels of experience to assess the activity status of lesions using visual inspection. They concluded that the experience of the examiners does not significantly influence the overall performance of visual inspection (Gimenez *et al.*, 2013a; Zandoná and Zero, 2006). Similar results were obtained with objective methods such as the DIAGNOdent (Bengtson *et al.*, 2005).

To investigate intra-examiner reproducibility, 50% of the sample was chosen randomly for re-examination after a period of 1-2 weeks. It was felt that re-examination of 50% was acceptable to capture the sample variation in lesions from sound to non-cavitated dentine lesions. The time interval of more than one week to re-examine the teeth was thought to be adequate to ensure that the observers could not recall previous results and to avoid any bias as a result of memory (Jablonski-Momeni *et al.*, 2010; Qudeimat *et al.*, 2015; Burin *et al.*, 2005).

All the teeth during the examinations were selected in a random order to avoid any systematic errors during examination sessions which could result from operator fatigue toward the end of the session or any errors due to teeth dehydrating for example.

In order to create a suitable environment that would replicate the clinical situation, all teeth were examined with the CarieScan PRO inside a sealed box fabricated for this purpose. Inside the box the teeth were kept and examined under standard conditions of humidity and temperature expected within the oral environment as variations in these factors could impact upon the results (Huysmons *et al.*, 2000). The tufted metal sensor was designed to be used only on occlusal surfaces at specific investigation sites: clinically it would be difficult to get the sensor proximally and was not recommended by the

manufacturer to be used for such purposes. As such proximal surfaces were not examined with this technique. All teeth were mounted inside a plastic mould where the roots were embedded in putty and surrounded with a conductive gel to avoid errors in readings due the movement of teeth during examination.

For all other caries detection methods, the posterior teeth were set in anatomical arches embedded in die stone with wood chips to mimic the clinical and radiographic appearance of bone. Care was taken to achieve appropriate anatomic relations including contact points. Anterior acrylic teeth were also add to create a realistic bilateral maxillary-mandibular relationship and proper contact points between the teeth which was particularly important for the radiographic examinations (Young *et al.,* 2009; Krzyzostaniak *et al.*, 2014; Cheng *et al.*, 2012).

The examiners were trained in the ICDAS system in a 2-hour session plus the 90 mim e-learning session, including theoretical and practical training on scoring teeth with a variety of ICDAS scores. In agreement with this a recent study suggested that short training online programmes would improve the performance and the reproducibility of the dentist using ICDAS for occlusal caries detection (Theocharopoulou *et al.,* 2015). However, the results of this study were in contrast to a previous study which found that the e-learning programme did not improve the performance of experienced dentists (Rodrigues *et al.,* 2013). The benefit of the e-learning ICDAS training program is therefore questionable, but it was felt that it would give the examiners the optimum amount and quality of training.

The modified criteria for FOTI was based on the classification suggested by Côrtes *et al,* (2000) and has been used by Gomez *et al,* (2013b) in a study investigating the performance of different methods for detecting occlusal lesions. In this study, the addition

of score 5 was done to represent extensive shadowing in dentine at the pulpal third for comparison with other classification systems such the one used for radiographic examination.

For all radiographic examinations, the maxilllary and mandibular arches were mounted using plastic hings to avoid streak artefacts in the Panoramic and CBCT images that would result from using metallic articulators (Benic *et al.*, 2013; Young *et al.*, 2009).

A soft tissue equivalent material was not placed in the beam path during the exposures of radiographic examinations to simulate scattered radiation and beam attenuation of oral soft tissues (Schropp *et al.*, 2012). This could influence the quality of radiographs in this study by artificially increasing the contrast of the images and improving detection. However, soft tissue equivalent application was difficult to apply for digital panoramic radiographs and CBCT and therefore was not applied to all radiographic modalities to standarised the images. This would also minimize the effect of contrast enhancement in the results to allow comparison between different radiographic methods (Da Silva *et al.*, 2010; Kamburoğlu *et al.*, 2010b). A previous study was also carried out by Koob *et al*, (2004) on the effect of digital filtering on the measurement of severity of proximal lesions under different exposure conditions with and without soft tissue equivelant material. The results did not reveal a statistically significant effect on the reproducibility or the overall accuracy of depth measurement.

All CBCT scans were performed at lowest possible voxel size of 0.125 mm and FOV of 4 x 16 cm to improve the contrast and resolution of the images. In a previous study, it has been shown that measurements made with CBCT were all comparable for voxel sizes up to 0.2mm despite a slight tendency towards underestimation, which increases significantly above voxel size of 0.3 mm (Maret *et al.*, 2012). Another study by

Kamburoğlu *et al.,* (2010b) compared 3D CBCT and intraoral imaging modalities for caries depth assessments using ultra-resolution and high- and low-resolution images with voxel size 0.1mm, 0.2mm and 0.3mm respectively. They showed that imaging modality performance was not different for deep enamel caries, superficial dentine and deep dentine caries for all voxel sizes .

One of the features of i-CAT imaging Cone Beam system used in this study is the ability to create two dimensional panoramic views which have been used in this study for the purpose of comparison with other (2D) images such as intraoral Digital Bitewing and Panoramic radiographs.

The assessment of inter-and intra-examiner reproducibility for all categorical data was carried out using Cohen's unweighted kappa and weighted kappa to remove the portion of agreement that would occur by chance. Both measurements were used in this study to allow comparisons with other studies as both have been used previously. It has been found that the magnitude of agreement could be affected by the prevalence of caries lesions at different severities and this should be borne in mind when comparing the results of this study with others (Banting *et al.*, 2011).

Bland and Altman plots were also used to look for systematic differences between and within examiners for the two examination techniques that used continuous data (DIAGNOdent Pen and CarieScan PRO), where in ideal cases there should be no systematic deviation (mean difference = zero) and only a small range between upper and lower limits of agreement (Rodrigues *et al.*, 2008).

2.5.1 Inter- and Intra-examiner Reproducibility for Occlusal Caries:

Regarding the frequency distribution of ICDAS and FOTI scores among all examiners for occlusal caries detection it is obvious that examiners were able to pick up early enamel lesions more than using other radiographic examinations. Moreover, radiographic examinations were able to pick up more shallow dentinal lesions. This is what would be expected as detection of early occlusal lesions is difficult even with radiographs due to the invaginated morphology and with the volume of sound buccal and lingual enamel and dentine which attenuates the x-ray beam (Krzyzostaniak *et al.*, 2014; Kidd *et al.*, 2003a; Galcerá Civera *et al.*, 2007). In addition, the amount of mineral loss that has to occur for lesions to be seen on a radiographs has been reported at about 30-40%, hence more extensive lesions are required to be detected on radiographs(Wenzel, 2004b; Wenzel, 2004a).

Concerning the digital DPT radiographs, only about half of the occlusal surfaces were scored and included in this part of the study. This is attributed to the inherent problems associated with DPT radiographs such as geometric distortion and considerable tooth overlap. Clinically, superimposition of adjacent neck and head structures and x-ray beam scattering effects would also affect the ability to determine caries lesions (Wyatt *et al.*, 1995; Tronje *et al.*, 1981).

The ICC values of DIAGNOdent Pen for inter- and intra-examiner reproducibility were 0.88 (0.86-0.91) and 0.83 (0.78-0.86) respectively, demonstrating excellent agreement between and within examiners. These findings are in agreement with other studies which achieved comparable results (Rodrigues *et al.*, 2011; Jablonski-Momeni *et al.*, 2012; Kühnisch *et al.*, 2007). Analysis of readings using limits of agreement described by Bland and Altman (1986) was used to look for systematic differences between inter- and intra-

examiner readings. Ideally there should be no systematic deviation when readings are repeated and the mean differences between readings should be zero with only a small range between the upper and the lower limits of agreement (Rodrigues *et al.*, 2008). Very little has been published in the literature for using this method of analysis in relation to the reproducibility of DIAGNOdent Pen. In our study, from the Bland and Altman plots the average mean difference for inter- and intra-examiner reproducibility was 0.1 and -0.7 respectively; close to the ideal and confirming no systemitic shift when readings are repeated. The average range between upper and lower limits of agreement however for inter- and intra-examiner reproducibility was 15 and 14 respectively. These findings are comparable with the results achieved by Lussi *et al*, (2006) where the mean difference for intra-examiner reproducibility was 0.31 and range of 9. It could also be observed in our study that the pairwise differences were smallest for the lowest values of fluorescence that is readings less than 15. Therefore differences in this order of magnitude for repeated readings must be borne in mind when monitoring early lesions over time.

When the DIAGNOdent Pen readings were converted into categorical data, the unweighted kappa values (SD) for inter- and intra-examiner agreement were 0.68 (0.02) and 0.71 (0.02) respectively, demonstrating a substantial agreement between and within examiners. The values whilst higher than those found by Rodrigues *et al*, (2008) (inter- and intra-examiner agreement were 0.58 and 0.60) they were lower than those found in *in vitro* studies by Lussi *et al.,* (2006) and Achilleos *et al*, (2013) in which the intra-examiner agreement (unweighted kappa) was 0.89 and 0.87 respectively and the inter-examiner reproducibility (unweighted kappa) 0.82 in the latter study. The teeth in the latter study were stored in distilled water and readings taken immediately after their extraction, this could explain the differences found in reproducibility as it has been shown

that different storage methods can affect the fluorescence measurements (Francescut *et al.*, 2006). The variations in the results between studies could also be due to types of lesions included and the cut-off points used to created the categorial data.

The ICDAS demonstrated a moderate to substantial inter- and intra-examiner agreement with mean weighted kappa values of 0.56 and 0.67 respectively. These findings agreed with those by Qudeimat *et al,* (2015) who investigated the reproducibility of ICDAS for detecting occlusal caries in permanent molars and the effect of variables on the reproducibility of ICDAS. They found inter-and intra-examiner weighted kappa values ranging between 0.42-0.72 and 0.66-0.81 respectively and concluded that the examiner's specialities and their clinical experience affected the reproducibility. In addition, our results are comparable with other studies where the agreement ranged between 0.51 and 0.66 for inter-examiner reproducibilty (Rodrigues *et al.*, 2013; Diniz *et al.*, 2009; Jablonski-Momeni *et al.*, 2008) and 0.58 and 0.76 for intra-examiner reproducibility (Rodrigues *et al.*, 2008; Diniz *et al.*, 2009; Jablonski-Momeni *et al.*, 2010).

The kappa values for both inter- and intra-examiner reproducibility for FOTI were (weighted kappa values (SD) 0.58 (0.07) and 0.67 (0.06) respectively) comparable to that obtained with ICDAS. Very little has been published in relation to the reproducibility of FOTI in the detection of occlusal caries (Ashley *et al.*, 1998; Wenzel *et al.*, 1992). However, our findings are lower than those reported in two studies by Cortes et al, (2000) and (2003) and one by Gomez *et al,* (2013b) where the intra-examiner reproducibiliy was 0.87, 0.78 and 0.91 respectively. The difference with our results may partly be explained by the differences in sample selection (our study aimed at detecting shallower non-cavitated lesions), different devices used and the experience of the examiners in using FOTI.

The inter- and intra-examiner reproducibility for CBCT detection of occlusal caries was moderate to substatial (mean weighted kappa 0.55 (SD = 0.10) and 0.58 (SD = 0.11) respectively) and slightly lower to those achieved by Alomari *et al,* (Alomari *et al.,* 2015) who compared different radiographic modalities for occlusal dentine caries detection (mean weighted kappa for inter- and intra-examiner agreement was 0.66 and 0.73 respectively). In another study using CBCT and phosphor storage plates to detect occlusal caries under amalgam restorations the intra-examiner reproducibility for CBCT was also found to be substantial (Kayipmaz *et al.,* 2011). In contrast, a recent study by Krzyzostaniak *et al,* (2014) looking at the accuracy of CBCT in the detection of non-cavitated occlusal caries lesions found a strong to very strong agreement between and within examiners. The better reproducibility in the latter study can partly be explained by the fact that the observers were dental radiologists with specialist experience in detecting carious lesions from radiographs. They also used a 5-point certitude rating scale rather than a classification system to differentiate between severity of lesions based upon depth.

Results achieved in this study for digital BW radiographs and occlusal caries were not encouraging with agreement between and within examiners being only fair in general (inter- and intra-agreement weighted kappa values (SD) 0.40 (0.10) and 0.41 (0.11) respectively). This reflects the difficulty examiners experienced in detecting and assessing the severity of lesions on the occlusal surface using BW radiographs. These findings were also found in a previous study by Soğur *et al.,* (2012) who aimed to determine the diagnostic accuracy using storage phosphor plates for occlusal caries detection in 72 non-cavitated permanent molar teeth. The examiners were 2 oral radiologists and 2 specialists in restorative dentistry, each with at least 5 years of experience in caries detection. They found that inter-observer and intra-observer

agreement for the detection of occlusal caries ranged between slight to substantial (range: 0.15–0.79). It could be that the specialists in restorative dentistry achieved the lower values when compered with the experience of the radiologists. Similar findings have also been reproted in a study comparing CBCT and storage phosphor plates where the inter-examiner agreement (weighted kappa value (SD) was 0.40 (0.09)) (Rathore *et al.*, 2012).

Panoramic radiographs (DPT) are the most frequently used extra-oral films in clinical practice despite their low diagnostic accuracy for caries detection (Bader and Shugars, 1993). No information in the literature regarding the reproducibility of panoramic radiographs for occlusal caries detection could be found. However, our results showed fair agreement between and among examiners for detecting lesions on the occlusal surface of those teeth scored; about half of the sample were deemed unsuitable to make a decision on due to tooth overlap. However, the panoramic view of the CBCT performed better than digital BW and the digital DPT in terms of agreement. The only paper found in the literature regarding the reproducibility of panoramic views generated from CBCT was published by Pittayapat *et al.,* (2013), where the authors aimed to compare subjective image quality of cone-beam CT (CBCT) panoramic reformatting with digital panoramic radiographs. Seven observers gave scores for overall image quality and visibility of 14 anatomical landmarks on four human dried skulls. They found that the inter-examiner agreement ranged from fair to substantial (weighted kappa 0.32–0.62) and intra-examiner agreement ranged from moderate to substantial (weighted kappa 0.46–0.74); caries unfortunatley was not one of the landmarks to be assessed.

The CarieScan PRO was inferior in terms of the inter- and intra-examiner reproducibility when compared to the results of other caries detection methods in this study. The ICC values (ICC (95% CI) for inter- and intra-examiner agreement were 0.33 (0.24-0.44) and

0.36 (0.25-0.48) respectively and the weighted kappa values (SD) were 0.32 (0.07) and 0.39 (0.08) respectively. These results demonstrated poor agreement for occlusal caries detection despite great efforts to standardise the examination technique and environment. There are few peer reviewed publications on the performance of AC impedance spectroscopy technique because CarieScan PRO is a relatively new and modern device for caries assessment; most reports have been abstracts or presentations at conferences. The findings of our study do not agreed with that by Mortensen *et al.,* (2014) who investigated the performance of CarieScan PRO and DIAGNOdent for occlusal caries detection of non-cavitated lesions. The ICC in that study for inter- and intra-examiner reproducibility of the CarieScan PRO was 0.47 and 0.78 respectively. These findings may be explained by the high caries prevalence in the sample and the limited number of examiners to demonstrate any discrepancy.

Bland and Altman plots for CarieScan PRO showed that the average mean difference for inter- and intra-examiner reproducibility was 7.9 and -2.6 respectively with the average range between upper and lower limits of 120 and 105. Figure 2.12 illustrates a diamond-shaped array of points representing better agreement with extreme measurements at the lower and upper ends of the impedance scale and greater variation for intermediate measurements. This phenomenon reflects a device output limitation effect and which is more prone to systematic errors which has also been observed before by Huysmans *et al,* (2005) when exploring technical problems associated with the reproducibility of electrical caries measurements. Our results would therefore indicate that the CarieScan PRO is unsuitable for monitoring carious lesions as the reproducibility is poor.

In general reproducibility is better for intra-examiner reproducibility as one is more likely to have greater consistency within one's self compared to another examiner; as such the

inter-examiner agreement on the occlusal surface will be the focus here. **Inter-examiner reproducibility** for all caries detection methods was presented after data was dichotomization, using specific cut-off points for each method, to demonstrate the reproducibility at D_1 diagnostic threshold (enamel and dentine lesions as caries) and D_3 diagnostic threshold (dentine lesions only as caries). This was done to complement the diagnostic accuracy results investigated and discussed in Chapter 4. The findings revealed that the performance of the DIAGNOdent pen was superior to the other methods at the D_1 threshold showing strong agreement between examiners when readings greater than 6 were classed as caries. Surprisingly, the DIAGNOdent Pen's reproducibility at the D_3 threshold was poor. This would indicate that the D_3 threshold used in this study (readings >17) was unsuitable in relation to reproducibility. The teeth used in this study were extracted before 2006 and stored in water which could have diluted and washed out any bacterial by products (which fluoresces and is the basis of DIAGNOdent readings) in the deeper more porous dentine lesions.

CarieScan PRO was the least reproducible examination method with poor agreement (kappa = 0.18) using the recommended cut-off reading of greater than 20 as indicating caries at the D_1 diagnostic threshold. However, the reproducibility at cut-off point greater than 90 used for the D_3 diagnostic threshold was moderate. A recent *in-vivo* and *in vitro* study by (Teo *et al.*, 2014) also found that the CarieScan PRO was inferior to ICDAS and DIAGNOdent Pen at the D_1 diagnostic threshold for occlusal caries detection on primary teeth. The kappa values were 0.42 at cut-off point >21 and 0.32 at cut-off point >50. At the D3 diagnostic threshold the agreement among the examiners was moderate at cut-off point >90 but in general there was poor agreement between the *in vitro* and *in vivo* results for the same teeth (Teo *et al.*, 2014). This would broadly agree with our findings,

however, the lower values obtained in our study at cut-off >20 could be explained by the lower mineral concentration and thinner thickness of enamel in primary teeth (Hueb De Menezes Oliveira *et al.,* 2010) when compared with permanent teeth. It also brings into question how reliable the laboratory readings and studies are.

The mean inter-examiner reproducibility (unweighted kappa) for ICDAS, FOTI and CBCT was 0.58, 0.52 and 0.57 at the D_1 diagnostic threshold and 0.62, 0.63 and 0.36 respectively at the D_3 diagnostic threshold, showing moderate agreement between examiners with the exception of CBCT at D_3 threshold which was fair. Our findings in relation to ICDAS (using threshold 2/3 as cut-off for dentine caries) are in agreement with a recent *in-vitro* study by El-Damanhoury *et al,* (2014) which aimed to assess the performance of freshmen dental students in comparison to dental graduates for occlusal caries detection before and after attending the ICDAS e-learning programme. The inter-examiner reproducibility for both groups at cut-off point 0/1 ranged between 0.53 and 0.81 before and after attending the e-learning programme of ICDAS. The inter-examiner reproducibility for both groups at cut-off point 2/ 3 ranged between 0.53 and 0.89 before and after attending the e-learning programme of ICDAS. Thus it would appear that the e-learning program which was also used in our study has the ability to improve inter-examiner reproducibility and would be of benefit for those thinking of using ICDAS in multi-centre clinical trials.

A further *in vitro* study by Silva *et al,* (2012) has also assessed inter-examiner reproducibility for occlusal caries detection in permanent teeth using ICDAS. The examiners were three graduate students and two undergraduate dental students without previous experience in the use of ICDAS. The inter-examiner reproducibility for both groups at cut-off point 0/ 1 ranged between 0.73 and 1.00 and at cut-off point 2/3 ranged

between 0.66 and 1.00. We can conclude from our study and those described above that ICDAS reproducibility is moderate for enamel and dentine lesions and that the variation among examiners seen could be explained by the differences in the clinical background and experience in the interpretation of the lesions.

The CBCT reproducibility was better at the D_1 (mean kappa = 0.57) diagnostic threshold compared to the D_3 threshold (mean kappa = 0.36). This moderate to fair agreement is in contrast to a recent *in vitro* study by Ertaş *et al.,* (2014) investigating the diagnostic accuracy of different radiographic imaging modalities including CBCT and PSP for detection of occlusal caries on permanent non-cavitated teeth. They found that the inter-examiner reproducibility for CBCT at D_1 and D_3 threshold 0.83 and 0.98 respectively showing excellent agreement between examiners. For PSP the inter-examiner reproducibility at the D_1 and D_3 thresholds were 0.65 and 0.81 respectively showing substantial agreement between examiners. The higher values obtained in the Ertaş *et al.,* (2014) study may be attributed to the number of examiners, where only two examiners are recruited one of them an oral radiologist who trained the other. Also, the different exposure settings and resolution of the images obtained in this study might explain part of the discrepancy between the findings.

2.5.2 Inter- and Intra-examiner Reproducibility for Proximal Caries:

In relation to the digital DPT radiographs, only about a third of the proximal surfaces were scored and included in this part of the study due to the inherent problems associated with proximal overlap. A previous study by Rushton *et al,* (1999) assessed the quality of panoramic radiographs in a sample of general dental practices. He found that about 33% were of poor quality and one of the reasons given is that the coloumn of vertebrae can

overlap around the proximal areas of premolars. As such this study confirms the guidelines that DPT radiographs should not be taken for caries detection alone.

ICDAS has mainly been used in previous studies on the occlusal surface, however in this study the kappa values for ICDAS on proximal surafces also demonstrates a substantial agreement between and within examiners; mean weighted kappa values were 0.62 and 0.7 respectively. Our findings are better than that of a recent *in vitro* study by Neuhaus *et al,* (2014) who looked at the accuracy of ICDAS for proximal caries detection on 120 permanent molar teeth, aligned in pairs to simulate approximal contact between teeth. In that study the intra-examiner agreement was lower (0.43). Our results for intra-examiner reproducibility are in agreement with the requirements of the ICDAS group which recommends a goal of a kappa value of at least 0.65 (Topping and Pitts, 2009) and those reported in an *in vitro* study to determine proximal lesions on primary molars where the examiners showed substantial agreement (Novaes *et al.*, 2009).

Very little has been published about the reproducibility of FOTI in the literature (Ashley *et al.*, 1998; Wenzel *et al.*, 1992). Our results for inter- and intra-examiner agreement (weighted kappa values (SD) 0.51 (0.09) and 0.59 (0.09) repectively) are however lower than those that have been published where reported FOTI weighted kappa values ranged between 0.65 and 0.79 indicating substantial agreement (Ashley *et al.*, 1998; Fennis-Ie *et al.*, 1998). This could be explained by the simpler classification system used in these studies which classified proximal surfaces as either sound, or as having an enamel shadow or dentine shadow; our study had five catergories to mirror more closely the radiographic scoring systems.

The only CBCT study in the literature reporting the reproducibility of proximal caries detection was by Kulczyk *et al.,* (2014) who investigated the effect of amalgam filling

artefacts on the detection of proximal lesions. In that study the inter-examiner kappa values were (unweighted kappa 0.49) in agreement with our results (unweighted kappa (SD) 0.49 (0.09)). However, the intra-examiner kappa value were (unweighted kappa 0.89) considerably higher than our results (unweighted kappa (SD) 0.53 (0.10)). This could be attributed to the examiners recruited in the Kulczyk *et al.*, (2014) study who were two experienced dental radiologists each with a minimum 5 years' experience.

The inter-examiner agreement for digital BW, DPT and PCBCT for proximal caries detection was lower (weighted kappa values (SD) 0.42 (0.07); 0.41 (0.09) and 0.35 (0.09) respectively) than the intra-examiner reproducibilty (weighted kappa values 0.55 (0.08); 0.53 (0.12) and 0.56 (0.09) respectively). Whilst the kappa values for BW and DPT radiographic methods showed moderate agreement between and within examiners, the inter-examiner reproducibility for PCBCT was inferior to the other examination methods.

The findings in this study regarding the inter-examiner agreement of digital BW is in agreement with a previous *in vitro* study on primary teeth where inter-examiner kappa values ranged between 0.46 to 0.67, however, the intra-examiner agreement values were higher than our values (kappa = 0.87-0.91) (Sogur *et al.*, 2011). This could be attributed to differences that exist in the experimental set-ups used and the fact that in the current study lesion assessment was performed using a scale relating to caries extent whereas in several other studies a 5-point confidence scale was preferred. Again, in a previous *in vitro* study by Mitropoulos *et al*, (2010) who compared ICDAS II with conventional and digital radiography for detection of non-cavitated caries on free proximal surfaces using Micro-CT as the validation method, the inter-examiner agreement obtained for digital BW was considerably higher than our study (weighted kappa 0.72). This could be

explained by the lack of proximal contacts which eliminate the possibility for proximal overlapping.

Our results showed that the performance of the examiners using PCBCT for detection of proximal lesions was lower than that when investigating lesions on DPT although, the proximal overlap on the DPT meant that only about a third of surfaces were scored with this method.

The ICC values for DIAGNOdent Pen for inter- and intra-examiner reproducibility were 0.36 (95% CI = 0.3-0.46) and 0.24 (95% CI = 0.14-0.36) respectively and weighted kappa values (SD) were 0.39 (0.02) and 0.32 (0.02) demonstrating a poor agreement between and within examiners for proximal caries detection. These findings are considerably lower than previous studies where the reproducibility of DIAGNOdentPen for proximal caries detection showed moderate to substantial agreement (De Souza *et al.*, 2014; Neuhaus *et al.*, 2014; Lussi *et al.*, 2006a); the variations in results can be attributed to the same reasons given for occlusal lesions.

The Bland and Altman plots for the DIAGNOdent Pen on proximal surfaces showed that the average mean difference in paired readings for inter- and intra-examiner reproducibility was 0.7 and -0.2 respectively and the average range between upper and lower limits of agreement was 12 and 14. These findings are comparable with the results achieved by Lussi *et al*, (2006) where the mean difference for intra-examiner reproducibility was -0.1, however, the average range between upper and lower limits was higher (mean = 32).

In an identical manner to that for occlusal caries the inter-examiner reproducibility for all caries detection methods was presented after data dichotomization, using specific cut-off

points for each method to demonstrate the reproducibility at the D_1 and D_3 diagnostic thresholds. The findings revealed that the performance of ICDAS was superior to the other detection methods at both the D_1 and D_3 threshold (unweighted kappa values (SD) 0.62 (0.10) and 0.63 (0.10) respectively) showing moderate to substantial agreement. The reproducibility of the examiners at D1 threshold using ICDAS is in agreement with a previous *in vitro* study by Shoaib *et al.*, (2009) who aimed to assess the reproducibility of the ICDAS on 112 primary molar teeth set up in groups of 4 to mimic their anatomical positions. The inter-examiner reproducibility at D_1 and D_3 threshold in that study was 0.7 and 0.66 respectively.

The performance of the examiners using CBCT at the D_1 diagnostic threshold (unweighted kappa values (SD) 0.59 (0.10)) was better than FOTI and BW (unweighted kappa value (SD) 0.49 (0.12) and 0.46 (0.09) respectively). However, the examiner reproducibility at the D_3 diagnostic threshold for CBCT and FOTI were similar (unweighted kappa value (SD) 0.64 (0.12) and 0.60 (0.09) respectively) and showed substantial agreement. This would be expected as FOTI is primarily used as an adjunctive method to visual examination for proximal lesions with dentine lesions' shadows being more obvious. Regarding, CBCT the examiners has the advantage of moving the cursor through the image slices and picking up dentine lesions more easily compared to the BW two dimensional images.

The DIAGNOdent and PCBCT inter-examiner reproducibility were inferior to other examination methods at both D_1 (unweighted kappa value (SD) 0.34 (0.14) and 0.36 (0.08)) and D_3 thresholds (unweighted kappa value (SD) 0.39 (0.10) and 0.33 (0.10)) showing poor agreement.

2.6 Conclusions

Within the limitations of the present study, the conclusions that could be drawn were:

1. The inter- and intra-examiner reproducibility of the DIAGNOdent Pen was superior compared with other examination methods for occlusal caries detection and showed strong agreement between and within examiners. On the other hand, the DIAGNOdent Pen was inferior to other examination methods for proximal caries detection showing poor agreement between and within examiners.
2. The CarieScan PRO was inferior to other examination methods for occlusal caries detection and had poor agreement between and within examiners.
3. The inter- and intra-examiner reproducibility of the ICDAS was superior compared with other examination methods for proximal caries detection having substantial agreement between and within examiners.
4. At D_1 diagnostic threshold for occlusal caries detection, DIAGNOdent Pen was superior to other examination methods; showed strong agreement between examiners. At D_3 diagnostic; ICDAS was superior to other examination methods; showed substantial agreement between examiners. The CarieScan PRO led to the poorest reproducibility at the D_1 diagnostic threshold and the DIAGNOdent Pen had the poorest reproducibility at the D_3 diagnostic threshold for occlusal caries detection.
5. At D_1 and D_3 diagnostic threshold for proximal caries detection, ICDAS was superior to other examination methods. The inter-examiner reproducibility showed substantial agreement between examiners. The DIAGNOdent Pen had the poorest reproducibility at D_1 diagnostic threshold and the Panoramic CBCT had

the poorest at the D_3 diagnostic threshold. In both methods, the inter-examiner reproducibility showed poor agreement between examiners.

CHAPTER Three

Comparison of Conventional Histology and Micro-CT for Validation of Occlusal and Proximal Caries on Permanent Teeth - A Qualitative Analysis

3.1 Introduction

To determine the accuracy of any detection method used for caries detection and classification, a comparison with a "gold standard" or validation technique is required. It is logical therefore that the validation technique, or "gold standard", be more accurate than the detection method. Regarding the validation of any caries diagnostic tool, histological examination has historically been regarded as the most superior method as it is capable of illustrating the true pathological status of tissues (Jablonski-Momeni *et al.*, 2009). Stereomicroscopic inspection of dental hard tissues (histological analysis) is the most widely-used "gold standard" for validation of caries detection methods. This can be performed using serial sections of teeth whose thickness can vary considerably, or simply teeth have been hemisected (Huysmans and Longbottom, 2004) . Although histological examination has been the main method of validation, it has some disadvantages, as it is a tissue-destructive method. Often areas of interest, for example the deepest aspect of the carious lesion can be destroyed during sectioning and further use of valuable tissue samples is not possible in future studies. Moreover, it does not allow for monitoring of an artificial carious lesion over time (Kawato *et al.*, 2009; Willmott *et al.*, 2007; Ricketts *et al.*, 1998; Wenzel *et al.*, 1991c).

Over the last three decades Micro-CT has been introduced as a novel digital technology that shows great promise for detecting subtle changes within structures (Davis and Wong, 1996). It can create three dimensional images, with high resolution in the range of 5-50µm, by using micro-focal spot x-ray sources and high-resolution, two-dimensional detectors. Hence, it has the potential to be a valuable alternative to routine histological examination without the need for associated destruction of teeth, maintaining them for testing new technologies in the future (Mitropoulos *et al.*, 2010; Huysmans and

Longbottom, 2004). To date, few studies have used Micro-CT to validate new caries detection methods (Mitropoulos *et al.*, 2010; Soviero *et al.*, 2012). In addition, few studies have been carried out to compare the images obtained by Micro-CT and histological sections.

To compare Micro-CT images and the corresponding histological sections, and to accurately relate these to the examination sites for validation purposes, requires a precise and reliable locating system. In order to accurately assign a specific examination site to a corresponding histological section when a tooth is serially sectioned, a novel technique has been described by (Jablonski-Momeni *et al.*, 2009). This technique used a right angled triangle of foil of known dimensions which was attached to the resected root surface of the tooth in question. On a clinical photograph of the occlusal surface with the foil beneath, a scale of known dimensions was superimposed and an X-Y co-ordinate was used to identify the examination site; the length of the foil at that investigation site could then be calculated and the section with that length of foil could be selected, accurately linking the examination site with corresponding histological section.

No known studies which compare histology and Micro-CT images have used such a system to ensure that corresponding Micro-CT and histological sections are selected; those that have been published simply compare sections and select pairs of images which appear to correspond and these studies have only been carried out on proximal surfaces and not on the more complex occlusal surface (Mitropoulos *et al.*, 2010; Soviero *et al.*, 2012).

This *in vitro* study therefore aims to evaluate the relationship between histological serial sections and Micro-CT images, for the assessment of the presence and depth of dental

caries on both the occlusal and proximal surfaces on permanent premolar and molar teeth. Accurate pairing of Micro-CT and histological images is fundamental to establishing this relationship. To achieve this aim a secondary aim of this study was to devise a novel technique based on that described by (Jablonski-Momeni *et al.*, 2009) and based around a composite resin right angled isosceles triangle. This is described in more detail in section 3.2.2 and 3.2.3.

3.2 Materials and Methods

3.2.1 Selection of Examiners:

Two examiners from the original eleven examiners evaluated the validation techniques used in this study. Both were qualified experienced dentists with at least 15 years post graduate experience; one was a Postgraduate student with an interest in Restorative Dentistry and the other was a Consultant in Restorative Dentistry with experience in caries diagnostic studies and histological validation.

3.2.2 Preparation of Novel Composite Triangle for Accurate Pairing of Micro-CT and Histological images:

Identical right angled isosceles triangles of composite measuring 11x11x15.5 mm and 1.5 mm thick were made for each tooth. To achieve this a "master" triangle was manufactured out of a polymer material (Full cure 720, Rutland Plastic Ltd., UK) using a computer aided design (CAD) and 3D printing rapid prototyping system (see later under Section 3.2.4). An impression of the "master" triangle was then taken in an addition cured silicone Lab putty material (Coltène/Whaledent Inc., Switzerland). This then acted as an index into which a radiopaque flowable SDR composite (DENTSPLY Limited, UK), with low

polymerisation shrinkage was placed and light cured to make composite triangles for each specimen tooth.

The composite triangle was placed under an oriented tooth sample. A picture of the tooth with the composite triangle below was taken and altered so that a triangular scale or grid (11 mm x 11 mm) fits directly over the composite. Previously selected investigation sites on the occlusal surface can now be given x and y-coordinates. Furthermore, another x-coordinate (x_1) can be identified which is the length of composite strip below the tooth at any given **y**-coordinate. When the tooth was sectioned along mesio-distal axis, starting at the buccal surface and working towards the lingual /palatal surface, serial sections were produced each with a '**strip**' of composite towards the apical aspect of the section. The length of this strip is x_1 and from this, the bucco-lingual/palatal position of the section can be identified. The mesio-distal position of the site within the section was identified by coordinate x_2. Thus, the exact location of any preselected occlusal site, in any histological section, and Micro-CT image can be identified and accurately paired (Figure 3.1).

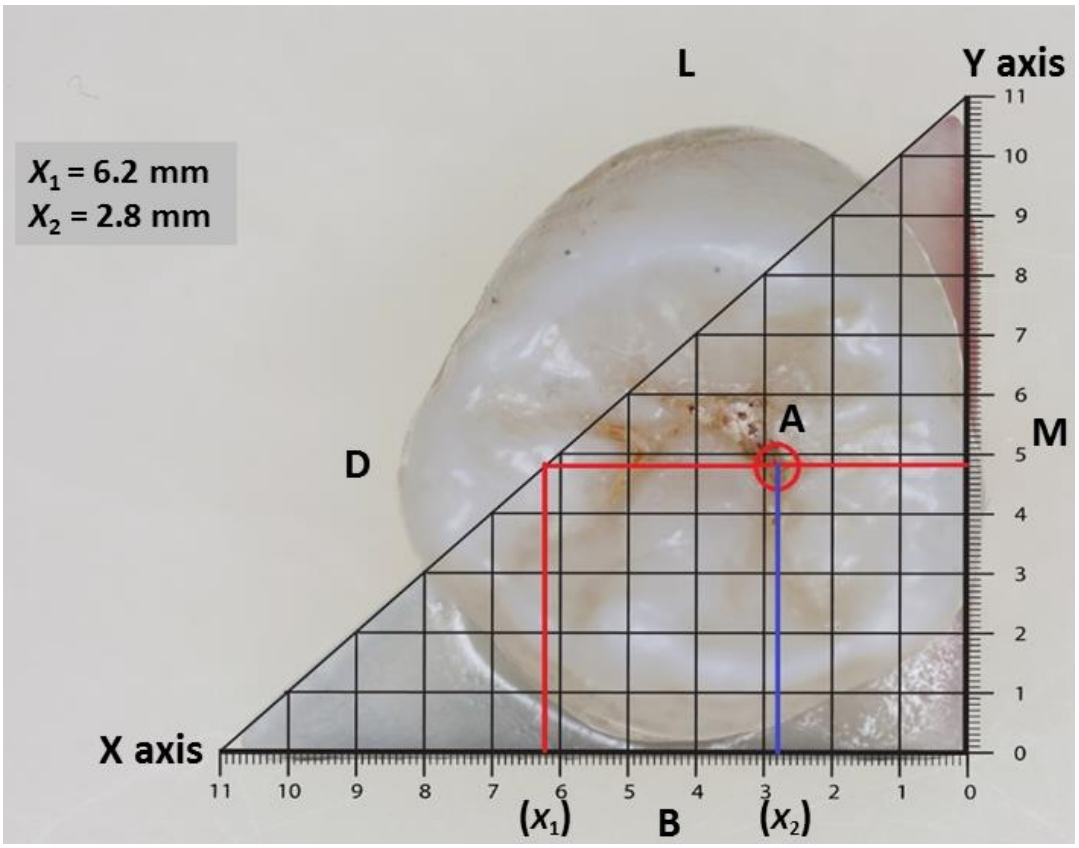


Figure 3.1 Photograph of the occlusal surface of a molar tooth with an 11x11x15.5 mm right angle triangular composite shape bonded to the resected root face, together with superimposed X- and Y- scales. Each point on the occlusal surface can be identified by two co-ordinates where (B, D, L and M denotes to buccal, distal, lingual/palatal and mesial surfaces respectively). For example, lesion A is located on **y** =4.9 mm when x_1 = 6.2 mm (i.e. when length of composite strip = 6.2 mm at plane **y** = 4.9 mm). However, there may be multiple lesions in mesio-distal plane, so x_2 = site of lesion along mesio-distal axis).

3.2.3 Tooth Preparation:

Prior to composite triangle bonding, the roots of each tooth were resected using a diamond bur with a horizontal cut parallel to the occlusal surface and just apical to the cement–enamel junction and the orientation groove on the mesial surface. A right angled composite triangle was then bonded onto the cut root face using Prime&Bond NT (DENTSPLY Limited, UK) with the mesial surface of the tooth (with cut groove on the root highlighted in red pen) aligned with one side of the triangle (Figure 3.1 and 3.2). The side of the triangle corresponding to the buccal aspect of the tooth was highlighted with

black pen. The red mesial mark on the tooth and the black buccal aspect of the composite triangle assisted in correct positioning in the embedding system and sample holder.

A photographic montage of the occlusal surface of each tooth attached to the right-angled triangular shaped composite was created to ensure that both were in focus. For this a Nikon D90 Digital SLR Camera equipped with Sigma 105mm f2.8 DG Macro lens and a Sigma DX8R ring flash was used and mounted on a tripod (Manfrotto 058B) and crossbar (Manfrotto 131) perpendicular to a light box (Jessops 1723). The sample was placed on the illuminated light box and the camera with the lens pre-set to a reproduction ratio of 1:1 was focused on the crown of the tooth. The first photograph was then taken and the whole camera assembly lowered using the tripod column drive until the midpoint of the tooth was in focus and another picture was taken. The assembly was then lowered further until the composite triangle base was in sharp focus and a final photograph was taken. The lens (front element) to occlusal surface distance was approximately 12cm.

The images were captured as camera RAW files and optimized and converted to TIFF format and displayed on a 17-inch monitor (Dell Computer Corporation, Austin, TX, USA). The three images of identical magnification were combined to produce one in focus image using Combine ZP software (Micropics, UK). Using the known dimensions of the composite triangle, X- and Y- co-ordinate scales were superimposed over the montage image of the tooth using paint.NET (dotPDN LLC., USA) (Figure 3.1). Each investigation site could therefore be assigned precise X- and Y- co-ordinates and the length of composite directly beneath the investigation site could be determined for use in selecting corresponding Micro-CT images and histological sections.

3.2.4 Fabrication of Sample Mould:

In order to scan each tooth using the Micro-CT, the tooth has to be mounted in a sample holder. The original sample holders used previously and provided by the manufacturer of the desktop μCT 40 Scanco system (Scanco MedicalAG, Bassersdorf, Switzerland) consisted of a series of cylinders of different dimensions in order to accommodate different sized samples and to image at different resolutions. Such cylindrical holders have no reference point to allow accurate orientation of the sample within the holder (and Micro-CT machine) and to allow tomographic images to be obtained in the correct and corresponding plane to that of the histological sections. To overcome this problem a custom formed system was devised to produce samples and sample holders with a square cross section area and a size that would fit within the dimensions of the original sample holders.

The components were initially designed by hand drawings and then transferred to a 3D format file (STL format file). The components of the **embedding system** and **sample holder** were made using a 3D printing rapid prototyping (Stereolithography) with high resolution (Connex printer, Rutland Plastics Ltd, UK).

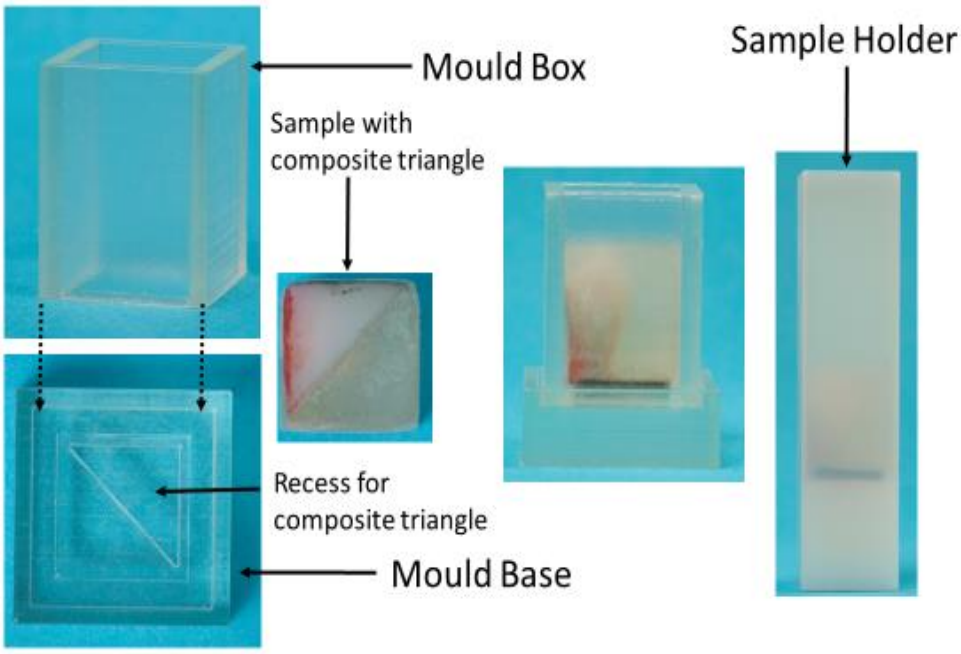


Figure 3.2 Custom formed system fabricated by 3D printing composed of mould base and mould box to embed the resected tooth with the composite triangle in acrylic, and a sample holder for mounting the sample inside the Micro-CT machine.

3.2.5 Micro-CT Examination:

One hundred and forty teeth, out of the entire set of 200 teeth used in Chapter 2, were scanned using a small-angle-cone-beam desktop μCT 40 Scanco system (Scanco MedicalAG, Bassersdorf, Switzerland) operated at 70 kVp and 114 μA, at a linear isotropic resolution of 20 μm. The integration time was set at 2.5 seconds, 500 projections taken over 180̊ (at 0.36° angle increments) and two frames per projection. The duration of the full scan time was dependant on the size of the examined teeth and ranged from 2 to 3.5 hours. For the scanning procedures, each embedded tooth was positioned in the sample holder, mounted on a computer-controlled turntable which allowed synchronized rotation and axial shift. A phantom consisting of five different density hydroxyapatite rods (0, 100, 200, 400 and 800 mg/cm3) was used to check the calibration of the system, on a weekly basis, throughout the study. Analysis of the mean and standard deviation

attenuation coefficient and density values of the hydroxyapatite rods was checked to ensure the stability of the x-ray tube. The accuracy of the beam-hardening correction was achieved by a 0.5mm aluminium filter which was installed in the beam path to cut off the softest x-rays resulting in a detector response close to 31 keV.

A hydroxyapatite disk of 2.2g/cm^3 was placed on the top of each embedded tooth sample before scanning for standardization of Micro-CT images and to allow quantitative assessment of mineral density of sound enamel, dentine and carious tooth tissues (see Chapter 5). Reconstructions of the images were carried out by cone-beam algorithms in 1024 x 1024 pixels matrices. All 1024 x 1024 pixels images were acquired in DICOM 16 bit images. Evaluation of the images was performed using **Image J** (public domain Java image processing program by NIH Image; see http://rsbweb.nih.gov/ij/appendix.html) on a 21 inch TFT computer screen.

In order to compare Micro-CT images with histological sections only tomograms in the sagittal (mesio-distal) plane were examined. The grey scale values corresponding to the background noise (embedding resin etc.) were eliminated from the Micro-CT images, isolating the voxels corresponding to the grey scale values of enamel and dentine structure. To further enhance lesion identification the contrast was set at approximately 20%.

The Micro-CT images of the teeth were viewed tomographic slice (20 μm) by tomographic slice until the slice that had the corresponding length of composite beneath the occlusal investigation site/s were found. This image slice was then optimised and stored in JPEG format for examination by the two examiners (independently and blindly) over a series of sessions to avoid examiner fatigue. The Micro-CT images corresponding to each occlusal investigation site was classified according to two subjective classification

systems namely the Downer (Table 3.1) and ERK (Table 3.2) systems. Where the depth of any occlusal lesion at each investigation site did not correspond to the deepest aspect of the lesion, the latter was also recorded together with the length of composite strip to enable accurate comparison between Micro-CT and histology images. For proximal surfaces the deepest aspect of the lesion (if present) only was recorded according to the two classification systems. Fifty per cent of the tomographic images were randomly selected and re-examined after 2-3 weeks to calculate intra-examiner reproducibility.

For each occlusal investigation site and proximal surface the results using each classification system from both examiners were then compared in order to achieve a consensus score for comparison with the corresponding histology scores and also to serve as a "gold standard" for validation of caries detection techniques (Chapter 4). Where disagreement between scores occurred, consensus was reached by re-examination and discussion.

score	Downer Histological Classification
0	Sound
1	Caries in the outer half of enamel
2	Caries in the inner half of enamel
3	Caries in the outer half of dentine
4	Caries in the inner half of dentine

Table 3.1 Downer Histological Classification Criteria (Downer, 1975).

code	ERK Histological Classification
0	Sound
1	Caries in the outer half of enamel
2	Caries in the inner half of enamel or extending to outer third of dentine
3	Caries in the middle third of dentine
4	Caries in the inner third of dentine

Table 3.2 ERK Histological Classification Criteria (Ekstrand *et al.*, 1997)

3.2.6 Histological Examination:

Once acceptable Micro-CT images had been achieved for 140 teeth, the entire sample of 200 embedded teeth was serially sectioned in a mesio-distal direction. For this, the blocks of embedded teeth were mounted on an ISOMAT® 5000 linear precision saw (Buehler Ltd., USA) and sectioned using a 250 µm thick circular diamond disc. The serial sections were between 250-350 µm thick.

All sections were glued to a glass slide alongside a piece of orthodontic wire of known dimension (used to calibrate all images) and digital images were obtained at 16x Magnification (Leica DC500 CCD camera, Leica Microsystems Digital Imaging, UK). The image of the section corresponding to each occlusal investigation site was selected based upon the length of the composite strip at the sectioned root face. Where the depth of the lesion (if present) beneath the investigation site did not correspond to the deepest aspect of the lesion, the image of the section with the latter was also selected for classification. For proximal surfaces the image of the section with the deepest aspect of the lesion (if present) was also selected for classification.

The selected images were then viewed by each examiner (independently and blind) on a 21 inch Thin Film Transistor (TFT) PC computer screen on separate sessions to classify

any caries according to the two subjective classification systems presented in Table 3.1 and 3.2. Caries identification was based upon colour or optical and structural changes in enamel and dentine, with emphasis being placed on differentiating carious changes from protective changes of the pulp-dentine complex, such as tubular sclerosis and tertiary dentine formation (Jablonski-Momeni *et al.*, 2008). The results of each classification system from both examiners, for each occlusal investigation site, the deepest aspect of occlusal lesion (if different), and each proximal surface were then compared in order to achieve a consensus score for comparison with the corresponding Micro-CT image and also to serve as a "gold standard" for validation of caries detection techniques (Chapter 4). Where disagreement between scores occurred, consensus was reached by re-examination and discussion. The corresponding fifty per cent of histological sections which were randomly selected for Micro-CT re-examination were also re-examined after 2-3 weeks to calculate intra-examiner reproducibility.

3.2.7 Statistical Analysis:

The weighed kappa statistic (Landis and Koch, 1977) was used to evaluate inter- and intra-examiner agreement for Micro-CT and histological examination for occlusal and proximal surfaces using each classification system for 140 teeth. All weighted kappa analyses were carried out using MedCalc V 13.1.2.0 statistical software (MedCalc Software bvba, Belgium), and the level of significance was set at $P < 0.05$. Cohen's unweighted kappa was also applied to assess inter- and intra-examiner reproducibility using SPSS software V 21(PASW statistics, SPSS Inc., Chicago, USA).

Descriptive statistics (frequencies) were used to summarize the frequency distribution of consensus scores given by Micro-CT and histological examination for both occlusal and proximal surfaces of 140 teeth, using the Downer and ERK classification systems, using

SPSS software V 21(PASW statistics, SPSS Inc., Chicago, USA) and Excel 2013 (Microsoft Corporation, USA).

The relationship between Micro-CT and histological examination for occlusal and proximal caries and both classification systems was examined using Spearman's correlation coefficient (r_s), using SPSS software V 21(PASW statistics, SPSS Inc., Chicago, USA). Percentage agreement between the histology and Micro-CT consensus scores was also calculated for both tooth surfaces using both classification systems. For these relationship analyses, any investigation sites which were damaged at sectioning were excluded and the sites where the deepest aspect of the lesion did not correspond with the investigation sites were included.

Using histology as the "gold standard" to validate the Micro-CT results, sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and accuracy (Ac) were calculated at the D_1 diagnostic threshold (any lesion in enamel or dentine classed as caries; cut-off between scores 0-1) and at the D_3 diagnostic threshold (only lesions in dentine classed as caries; cut-off between scores 2-3). For this the data was converted into dichotomous data by using the Downer classification system only to calculate these values at both the D_1 and D_3 diagnostic thresholds. This was carried out for both the occlusal and proximal surfaces using all of the occlusal investigation sites including the sites representing the deepest lesion (n=179) and proximal surfaces (n=280) examined, including the investigation sites where damage had occurred. Where section damage occurred and this involved loss of enamel only from the dentine and no dentine/lesion damage, it was possible to say whether there was a dentine lesion present or not and hence allow calculation of values at the D_3 diagnostic threshold. Using consensus data (Appendix 2.1) collected from the preliminary inspection of the hand-

held, clean, dry teeth under ideal lighting conditions it was possible to say whether the investigation sites/surfaces were sound or whether there was any signs of caries. Using this data it was possible to determine sensitivity values etc. at the D_1 diagnostic threshold.

3.4 Results

A total of 140 teeth were examined with both the Micro-CT and histology and only the results pertaining to this sub-set of teeth will be considered further in this Chapter, despite the fact that a further 60 teeth were examined clinically and with histology (Chapter 2 and 4).

In total there were 172 investigation sites on the occlusal surface and 280 proximal surfaces examined. On the occlusal surface one of the examination sites represented the deepest aspect of the lesion for that tooth in 96% of cases. In 7 teeth the deepest aspect of the occlusal lesion was at a different site and this location was recorded using the length of the composite triangle for further comparison between the Micro-CT and histology making a total of 179 occlusal investigation sites. During sectioning for histological examination 8 occlusal investigation sites (5%) and 51 proximal surfaces (18%) were lost due to section destruction. This left 171 occlusal investigation sites and 229 proximal surfaces for examination. Where section damage occurred this involved loss of enamel only from the dentine and no dentine/lesion damage. A flow chart representing the procedure for Micro-CT and histology is illustrated in Figure 3.3.

An example of Micro-CT images and the corresponding histological sections of consensus Downer classification for occlusal and proximal lesions is illustrated in Figure 3.4 and 3.5 respectively.

The inter- and intra- examiner weighted and unweighted kappa values for the Micro-CT and histological scoring for occlusal and proximal caries using the Downer and the ERK scoring systems can be seen in Table 3.3.

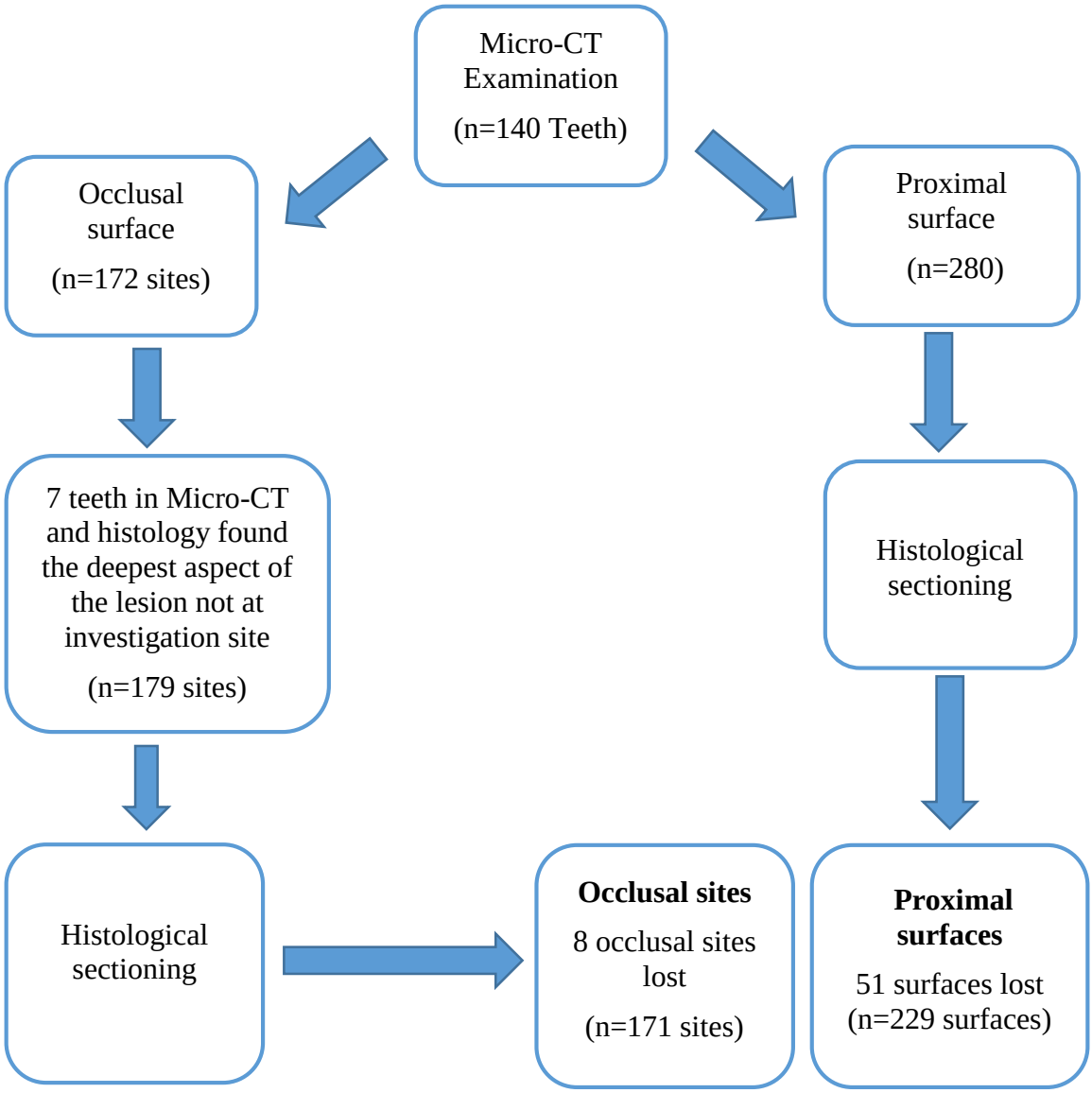


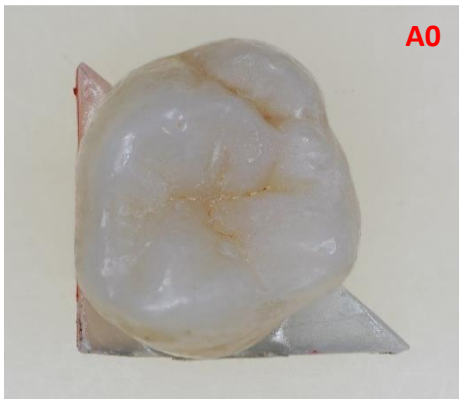
Figure 3.3 Flow chart representing the validation procedure using Micro-CT and histology.

		Inter-examiner Reproducibility		Intra-examiner Reproducibility			
				Ex 1		Ex 2	
		Downer	ERK	Downer	ERK	Downer	ERK
Occlusal N=171	Micro-CT	0.82 (0.72)	0.79 (0.72)	0.92 (0.84)	0.90 (0.85)	0.93 (0.84)	0.94 (0.86)
	Histology	0.94 (0.88)	0.95 (0.87)	0.94 (0.88)	0.91 (0.84)	0.96 (0.89)	0.95 (0.87)
Proximal N=229	Micro-CT	0.98 (0.91)	0.98 (0.92)	0.98 (0.91)	0.98 (0.91)	0.97 (0.91)	0.98 (0.90)
	Histology	0.97 (0.90)	0.97 (0.91)	0.94 (0.89)	0.95 (0.86)	0.95 (0.90)	0.93 (0.86)

Table 3.3 Summary of inter- and intra- examiner weighted and unweighted kappa values for the Micro-CT and histological scoring using the Downer and the ERK scoring systems; unweighted kappa is in parenthesis; N= is the number of occlusal investigation sites and proximal surfaces.

With exception of the inter-examiner reproducibility of occlusal caries with Micro-CT using ERK classification system, all the results for reproducibility indicated very strong agreement between and within examiners.

The frequency distributions of both the Downer and ERK consensus scores for occlusal investigation sites and proximal surfaces determined from Micro-CT and histological examination is presented in Figure 3.6 and Figure 3.7 respectively.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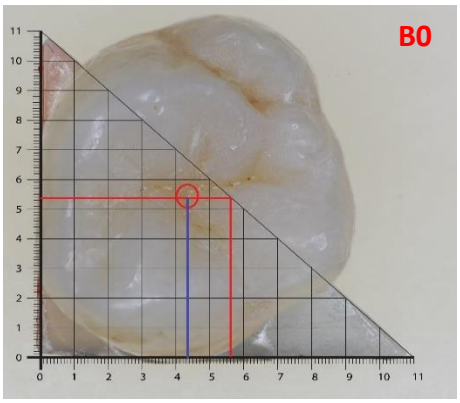
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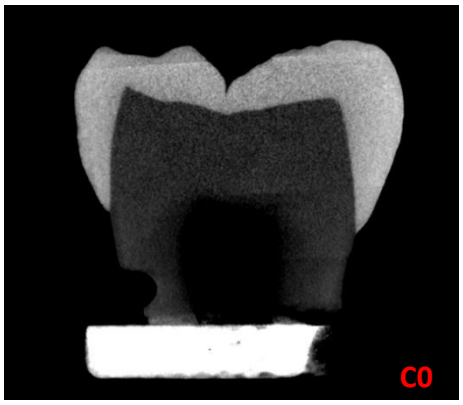
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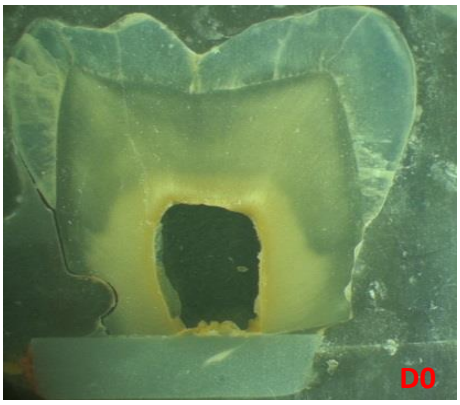
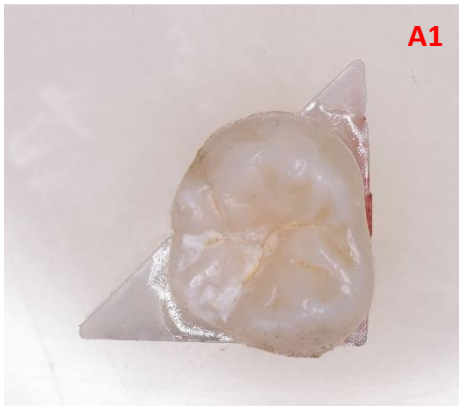

D0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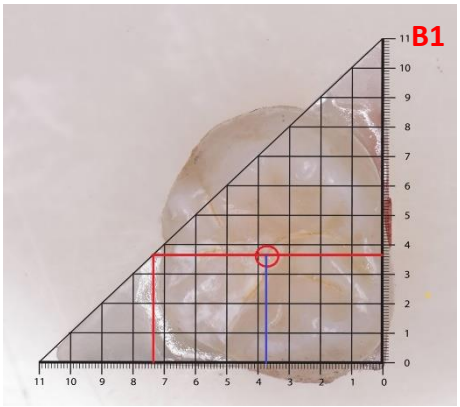
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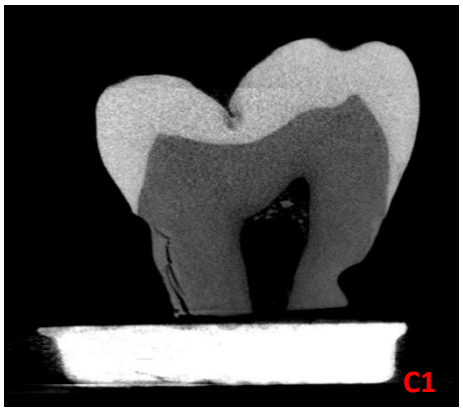
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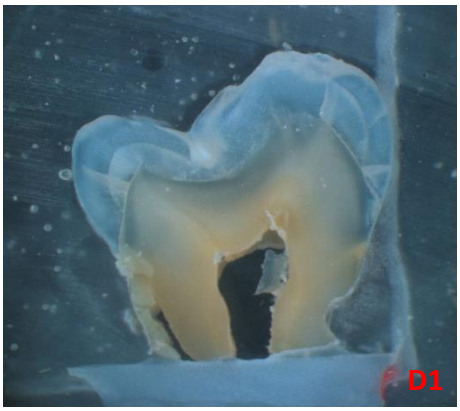
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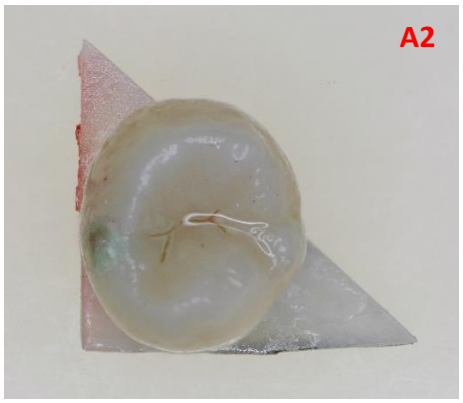
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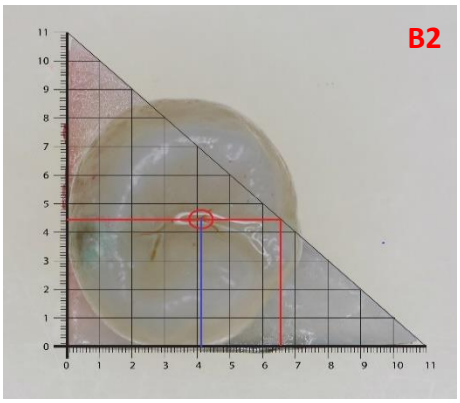
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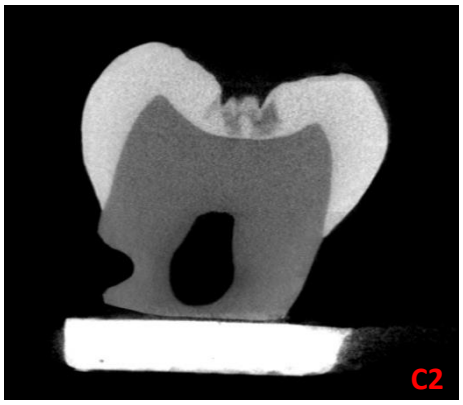
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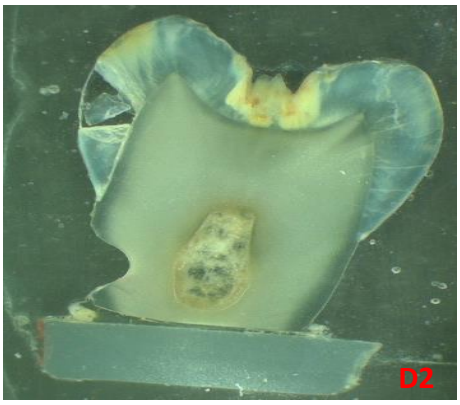
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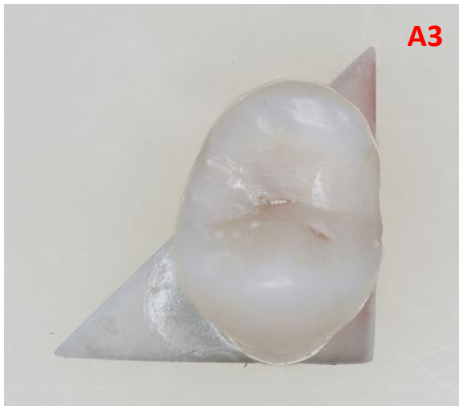
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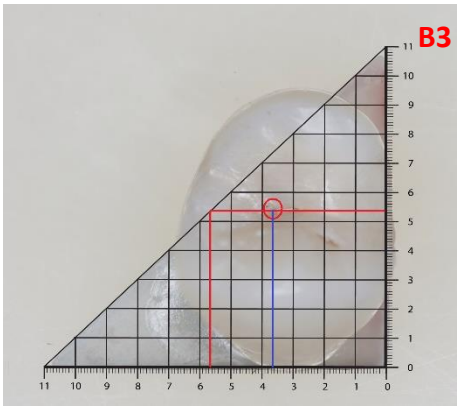
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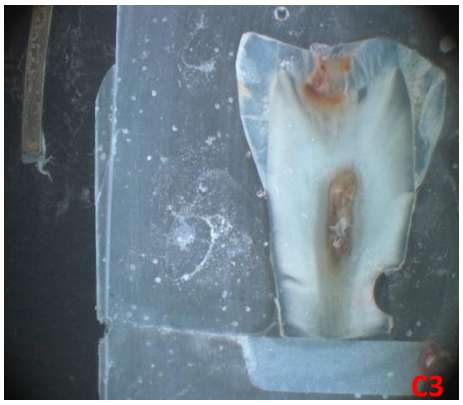
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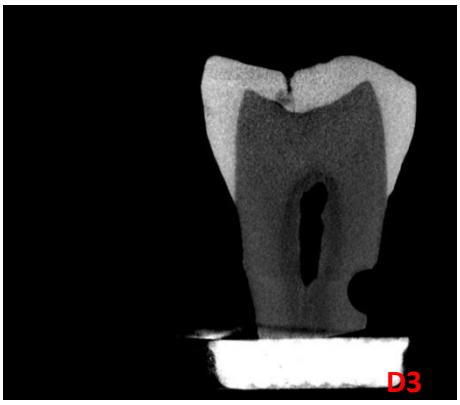
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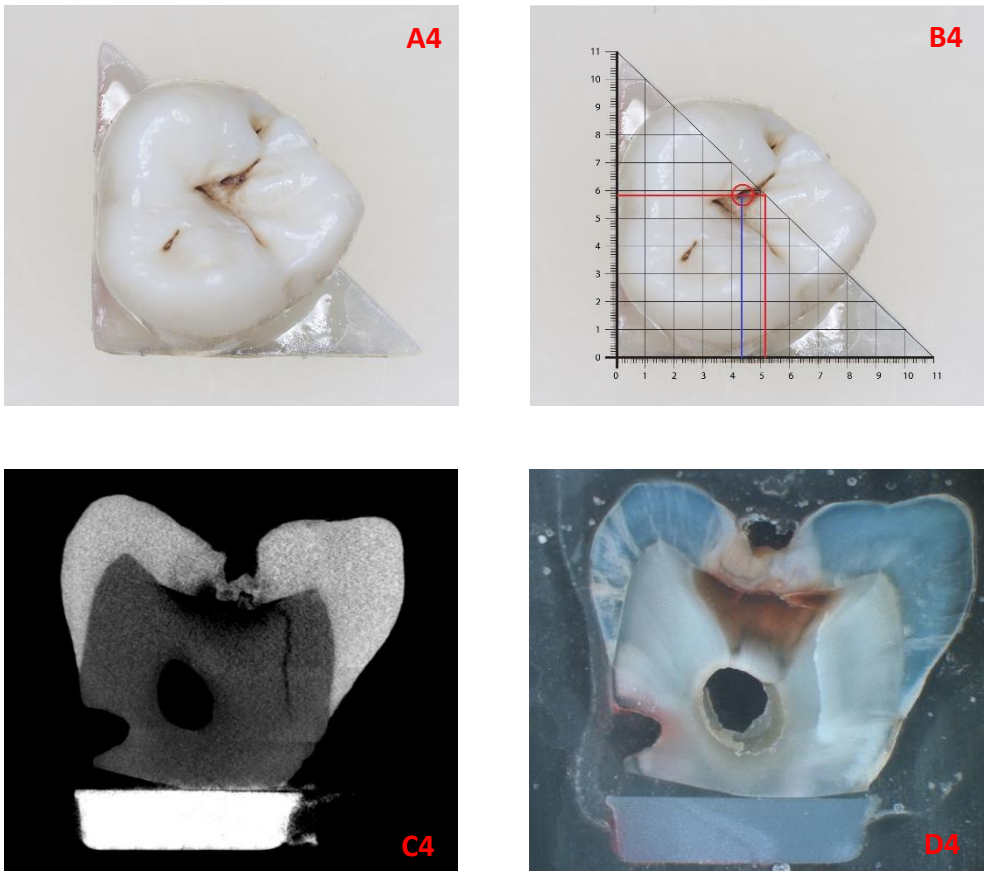

Figure 3.4 A group of images including a photograph of an occlusal surface of a tooth with 11x11x15.5 mm right angle isosceles triangle of composite bonded to its resected root (A) together with a marked preselected investigation site (B), the corresponding tomographic image (C) and the histological section (D) scored using Downer classification system score 0, 1, 2, 3 and 4 respectively; (C3) shows a piece of orthodontic wire of known dimension (used to calibrate all images).

Cross-tabulation of the consensus Micro-CT and histology results using the Downer and ERK classification systems for the 171 occlusal investigation sites and 229 proximal surfaces can be seen in Table 3.4 and 3.5 respectively.

The Percentage agreement between the Micro-CT and histology consensus scores for proximal caries using Downer and ERK classification systems was higher than that for occlusal caries. However, a strong direct relationship existed between Micro-CT and histology for detection of both occlusal and proximal caries using Downer and ERK classification systems.


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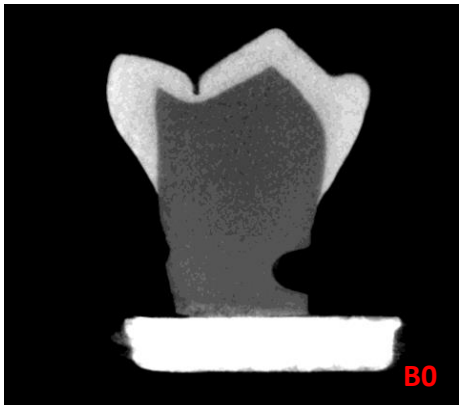
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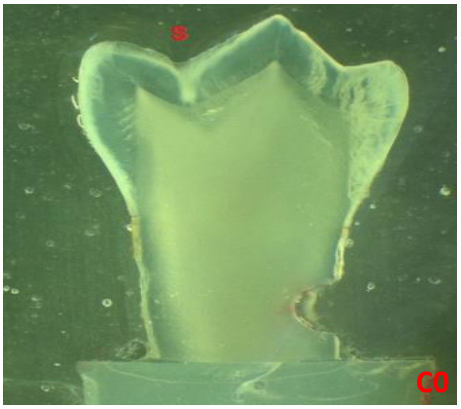
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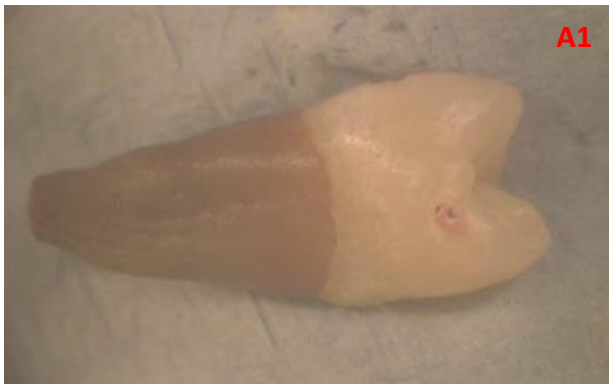
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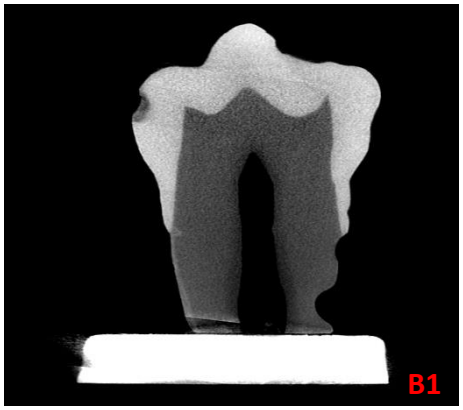
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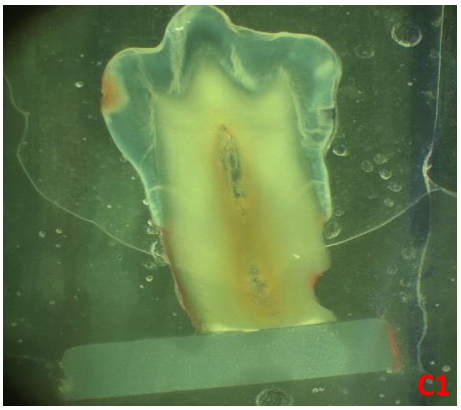
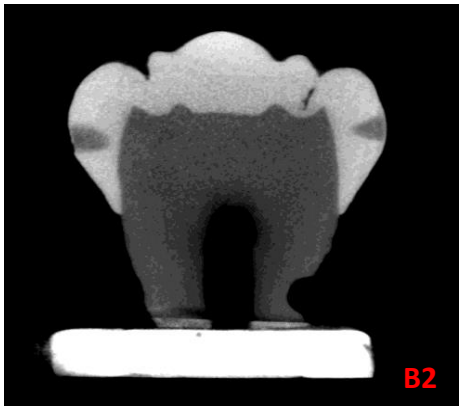

C1


A2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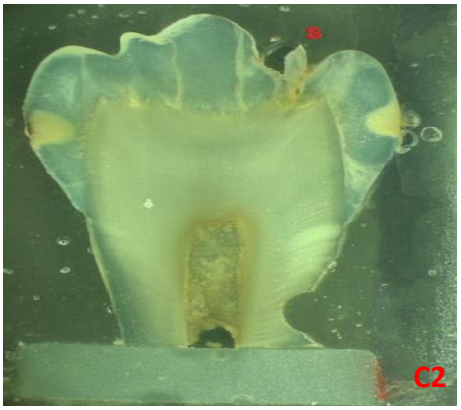
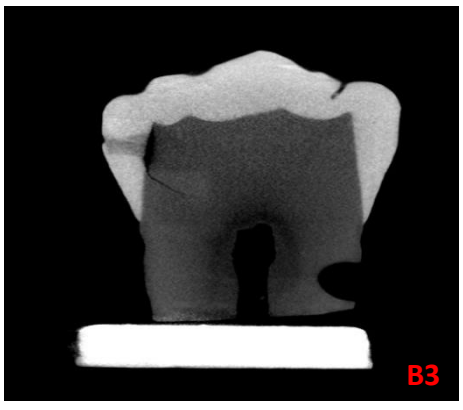

C2


A3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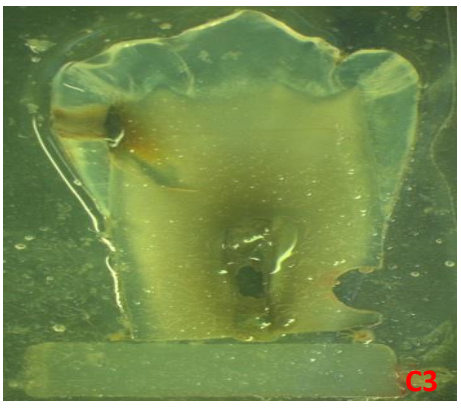
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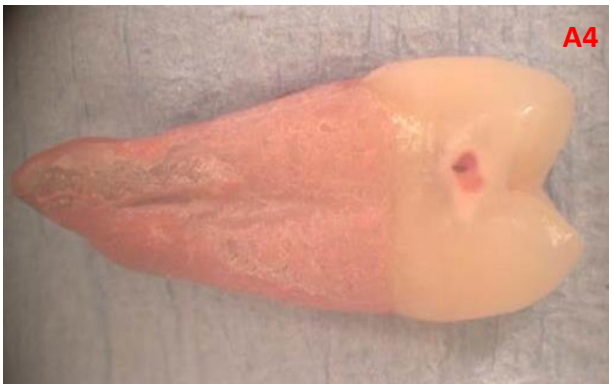
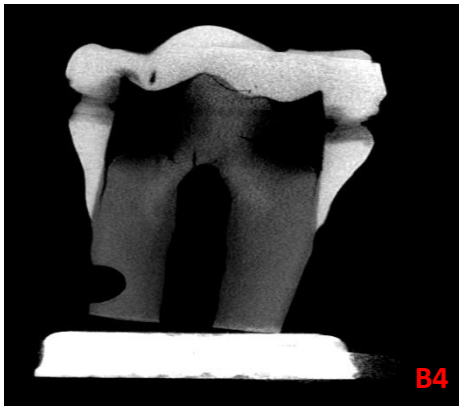




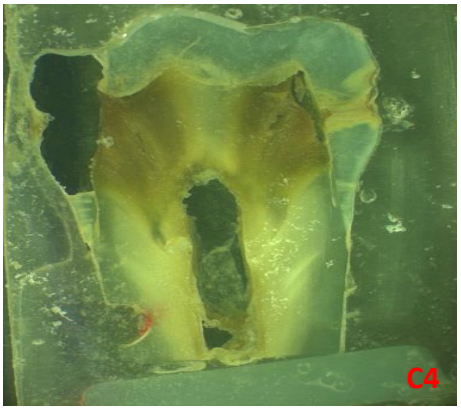
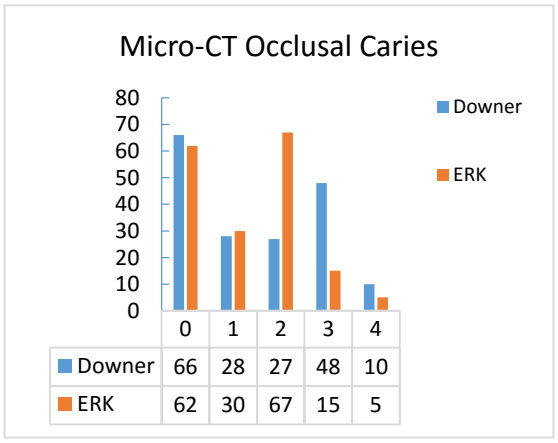


Figure 3.5 A group of images including showing the proximal surface under investigation (A), the corresponding tomographic image (B) and the histological section (C) scored using downer classification system score 0, 1, 2, 3 and 4 respectively.



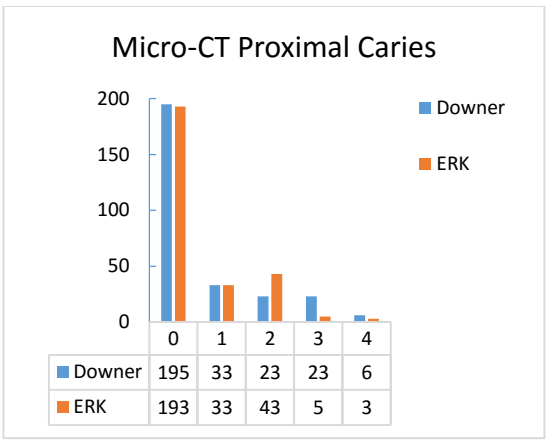
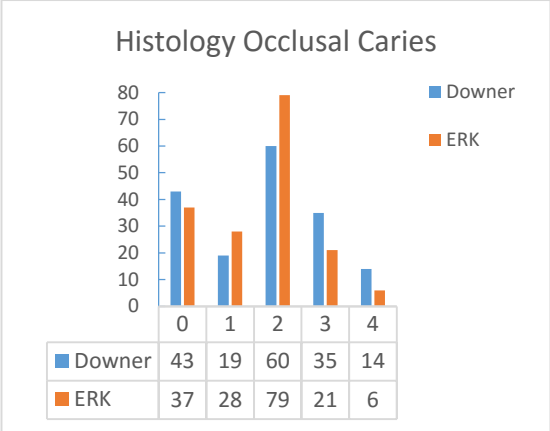


Figure 3.6 Frequency distribution of Downer and ERK **Micro-CT** consensus scores for 179 occlusal investigation sites and 280 proximal surfaces



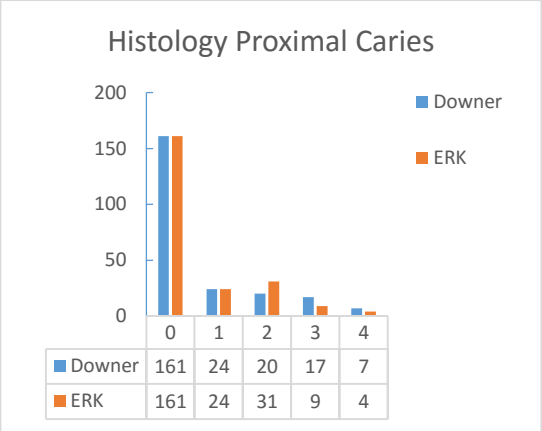


Figure 3.7 Frequency distribution of Downer and ERK **histology** consensus scores for 171 occlusal investigation sites and 229 proximal surfaces.

A		Consensus Histology for occlusal caries (Downer)					Total
		0	1	2	3	4	
Consensus Micro-CT for occlusal caries (Downer)	0	**39**	10	16	0	0	65
	1	3	**9**	14	1	0	27
	2	0	0	**22**	4	0	26
	3	0	0	9	**30**	4	43
	4	0	0	0	0	**10**	10
Total		42	19	61	35	14	171
Percentage agreement = 61%; Spearman rank correlation coefficient = 0.85; P< 0.001							

B		Consensus Histology for occlusal caries (ERK)					Total
		0	1	2	3	4	
Consensus Micro-CT for occlusal caries (ERK)	0	**35**	17	9	0	0	61
	1	2	**11**	15	1	0	29
	2	0	0	**55**	6	0	61
	3	0	0	0	**12**	3	15
	4	0	0	0	2	**3**	5
Total		37	28	79	21	6	171
Percentage agreement = 68%; Spearman rank correlation coefficient = 0.84; P< 0.001							

Table 3.4 Crosstabulation of consensus Micro-CT and histology for 171 occlusal investigation sites using Downer (A) and ERK (B) classification systems.

A		Consensus Histology for proximal caries (Downer)					Total
		0	1	2	3	4	
Consensus Micro-CT for proximal caries (Downer)	0	**152**	3	4	0	0	159
	1	6	**20**	5	0	0	31
	2	2	1	**10**	0	0	13
	3	1	0	1	**15**	3	20
	4	0	0	0	2	**4**	6
Total		161	24	20	17	7	229
Percentage agreement = 88%; Spearman rank correlation coefficient = 0.86; P< 0.001							

B		Consensus Histology for proximal caries (ERK)					Total
		0	1	2	3	4	
Consensus Micro-CT for proximal caries (ERK)	0	**154**	2	3	0	0	159
	1	5	**20**	4	0	0	29
	2	2	2	**23**	5	1	33
	3	1	0	0	**3**	1	5
	4	0	0	0	1	**2**	3
Total		162	24	30	9	4	229
Percentage agreement = 88%; Spearman rank correlation coefficient = 0.86; P< 0.001							

Table 3.5 Crosstabulation of consensus Micro-CT and histology for 229 proximal surfaces using Downer (A) and ERK (B) classification systems

Assuming that the histology is the most accurate of the two validation techniques and is used as the "gold standard" to validate the Micro-CT, sensitivity (Se), specificity (Sp), accuracy (Ac), positive predictive value (PPV) and negative predictive value (NPV) were calculated at the D_1 and D_3 diagnostic threshold using the Downer classification. This was done separately for occlusal and proximal caries for 140 teeth (179 occlusal investigation sites and 280 proximal surfaces) using the consensus scores. This included the additional deepest sites that represented the deepest aspect of a lesion and sites where

section damage had occurred, using the method described in section 3.2.7. A summary of this data is represented in Table 3.6.

	D_1 Diagnostic Threshold		D_3 Diagnostic Threshold	
	Occlusal	**Proximal**	**Occlusal**	**Proximal**
Cut-off point	**0/1**	**0/1**	**2/3**	**2/3**
Sensitivity %	88%	91%	91%	98%
Specificity %	93%	95%	93%	99%
Accuracy %	89%	94%	92%	99%
Positive Predictive Value %	97.5%	88%	84%	93%
Negative Predictive Value %	76%	96%	96%	100%
Prevalence of disease %	76%	29%	30%	10%

Table 3.6 Summary of sensitivity (Se), specificity (Sp), accuracy (Ac), positive predictive value (PPV) and negative predictive value (NPV) for occlusal and proximal caries validation of Micro-CT assuming histology as the "gold standard" using Downer classification system at the D_1 and D_3 diagnostic threshold.

With the exception of the negative predictive value for occlusal caries at the D_1 diagnostic threshold, all results on the performance of Micro-CT derived from the dichotomised data were greater than 84%, demonstrating a high degree of accuracy compared with histology.

3.5 Discussion

The outcome of any detection method must be compared against the true status of the presence or absence of disease using a validation or reference method also called a "gold standard". Not only is diagnostic accuracy important but the reproducibility should also be high (Wenzel and Hintze, 1999). Conventionally, 'gold standards' used in the evaluation of different caries detection methods are based on histological examination

using a stereomicroscope or light microscope (Jablonski-Momeni *et al.*, 2009; Huysmans and Longbottom, 2004; Hintze *et al.*, 1995). However, there is no absolute validation method to determine the real extension or mineral loss of any carious lesion as the investigation of tooth sections using different validation methods, under different conditions could yield different outcomes (Rodrigues *et al.*, 2012). Moreover, it is difficult or even impossible to validate most gold-standard evaluation method(s) (Wenzel *et al.*, 1994), so it is more valuable where possible to rely on the results of different gold standards for the purpose of comparison of caries detection methods.

Sectioning teeth for histological examination is destructive, and requires significant time and skilled personnel. In addition, it is difficult to assess the three dimensional nature of the lesion from a single cut surface (Kawato *et al.*, 2009). Where the section cut occurs, tooth tissue loss occurs as the tooth is ground down. This may lead to the loss of the deepest part of the lesion under investigation. It is also possible to cause catastrophic damage of the dental tissues during sectioning and loss of the tooth for further evaluation, as found in this study (see below). In cariology research there is, therefore, an increased demand for a non-destructive technique which will not only simplify the investigation procedure but also allow for the preservation of the sample for further use in diagnostic studies and for monitoring of artificial lesions (Ricketts *et al.*, 1998).

Micro-CT has been introduced as a novel digital technology which allows a non-destructive visualization of subtle changes within dental structures (Swain and Xue, 2009). In dentistry, Micro-CT has been used to observe the structure of bone, measure enamel thickness and to study the morphology of carious lesions (Hayakawa *et al.*, 2000) and through examination of multiple sections to determine the actual depth of carious lesions (Mitropoulos *et al.*, 2010). It has also been used in studies evaluating the internal

structure and mineral concentration of the human teeth (Wong *et al.*, 2006; Dowker *et al.*, 2003).

The main aim of this part of the study was to compare Micro-CT and histology for possible use as a "gold standard" in *in vitro* studies investigating the presence and depth of dental caries on both the occlusal and proximal surfaces on permanent premolar and molar teeth. Accurate pairing of Micro-CT tomographic images and histological images is fundamental to establishing this relationship. To achieve this aim a secondary aim of this study was to devise a novel technique based on that described by (Jablonski-Momeni *et al.*, 2009) which used a foil right angled isosceles triangle. In this study a composite material was used for fabricating the triangles as this material adhered well to the tooth structure maintaining it in place before and during the embedding in acrylic resin process. The composite material was also radiopaque making it easier to measure the strip of composite in the Micro-CT images as well as in the histological sections. The process of identifying the preselected occlusal investigation site by superimposing the scale with the x and y- coordinates, simplified the investigation site identification process and made pairing of identical histological sections with Micro-CT tomographic cuts much more reliable.

A commercial desktop µCT 40 Scanco machine based in Queen Mary University of London was used for scanning the teeth. This machine has been used previously in *in-vitro* studies to observe biogenic hard tissues such as bone, to determine mineral density (Nazarian *et al.*, 2008), to compare different caries excavation techniques (Lai *et al.*, 2014) and also tooth to investigate the effect of different materials on de-and re-mineralisation of enamel and dentine (Zhi *et al.*, 2013). For this study, a custom formed system was devised using three dimensional prototyping to allow construction of an

embedding system and a rectangular sample holder to accommodate tooth specimens in an accurate orientation within the holder to allow tomographic images to be obtained in a mesio-distal direction consistent with corresponding histological sections.

One hundred and forty teeth out of the entire sample of 200 were scanned and the results were used for comparison with histological sections. The duration of scanning for each specimen varied according to the size of the tooth and reached a maximum of 3.5 hours, this was followed by a reconstruction of the images which took a further one hour. Time is therefore one of the drawbacks of using Micro-CT where long scanning time and image reconstruction is needed and may reach nine hours at higher resolutions (Kamburoglu *et al.*, 2011; Davis and Wong, 1996). For this reason only 140 teeth were scanned in this study. To the best of the author's knowledge, this is the first study that has included such a large sample size for Micro-CT to investigate lesion depth on occlusal and proximal surfaces (Table 3.7).

Serial sectioning of extracted teeth is commonly carried out for histological examination and used as a gold standard for validation of conventional and new diagnostic modalities (Rodrigues *et al.*, 2012; Kawato *et al.*, 2009; Jablonski-Momeni *et al.*, 2008). Subsequent to the Micro-CT the 140 teeth were serially sectioned to enable the newer validation method (Micro-CT) to be compared to the traditional (serial sectioning) as both allowed visualisation of the tooth and lesion in mesio-distal planes. Both methods also allowed measurement of the length of the composite triangle in corresponding images to enable identical pairing of images.

Two histological classification systems (Downer and ERK) were used in this study to determine lesion depth on occlusal and proximal surfaces from both the histological sections and tomographic images. Both systems have been used previously for validation

of ICDAS II (Jablonski-Momeni *et al.*, 2008; Shoaib *et al.*, 2009) and in another study to determine the relationship between different histological techniques when validating the ERK visual classification system (Kidd *et al.*, 2003b). Both systems have also been used individually in studies on caries detection (Teo *et al.*, 2014; Mortensen *et al.*, 2014; Erten *et al.*, 2005; Parviainen *et al.*, 2013; Achilleos *et al.*, 2013) and inclusion of both in this study allows comparison of the results with studies which have used either system. The use of the Downer classification also allows diagnostic accuracy to be investigated at both the D_1 and D_3 diagnostic threshold as it uses the EDJ as a cut-off between codes 2 and 3. The ERK histological classification does not use the EDJ as a threshold as it was developed primarily to identify those lesions extending beyond EDJ by one third of the dentine thickness where lesions can still be treated preventively, hence avoiding the notion of the EDJ as a threshold for operative treatment (Ekstrand *et al.*, 1997).

One of the limitations of using histology for validation of dentinal caries is the difficulty in determining the pulp-ward extent of the lesion. In the past, emphasis has been placed on differentiating carious changes from protective changes of the pulp-dentine complex, such as tubular sclerosis and tertiary dentine formation (Banerjee, 2000; Bjørndal *et al.*, 1998; Six *et al.*, 2000). Micro-CT images on the other hand can detect any mineral decalcification/loss within a lesion accurately (Hahn *et al.*, 2004; Clementino-Luedemann and Kunzelmann, 2006). Micro-CT therefore has the potential to be a better way for defining the boundaries and extent of lesions as it is a less confusing and subjective assessment. Furthermore, Micro-CT lends itself to objective analysis (see Chapter 5).

In a literature search undertaken using the Scopus search engine for qualitative studies using Micro-CT in relation to dental caries validation, eight studies were found and summarised in Table 3.7. These studies have shown that Micro-CT is an acceptable

validation method for various detection methods including digital and conventional radiography (Matsuda *et al.*, 2002), CBCT and CCD systems (Tsuchida *et al.*, 2007; Young *et al.*, 2009; Kamburoglu *et al.*, 2011), ICDAS II (Mitropoulos *et al.*, 2010; Soviero *et al.*, 2012), and DIAGNOdent device (Kawato *et al.*, 2009). Whilst there have been a number of abstracts or presentations at conferences comparing histology and Micro-CT there were only four studies that have been published in peer reviewed journals (Mitropoulos *et al.*, 2010; Soviero *et al.*, 2012; Kawato *et al.*, 2009; Kamburoglu *et al.*, 2011). These studies investigated between 20 to 27 teeth, two of which looked at occlusal caries only and two proximal caries.

In this study only 7 occlusal investigation sites out of 171 did not reflect the deepest lesion within the tooth, which means that the preliminary inspection to determine the worse affected site failed in only 4% of cases. This small discrepancy is unlikely to impact upon the comparison of the diagnostic accuracy of "site specific" caries detection methods i.e. ICDAS, FOTI, DIAGNOdent Pen and CarieScan PRO and "overall" caries detection methods i.e. BW, CBCT and PCBCT radiographic methods (see Chapter 4).

During sectioning for histological examination, only 5% of occlusal investigation sites were lost due to section destruction, however, 18.5% of proximal surfaces were lost, a problem highlighted in previous publications (Ricketts *et al.*, 1998; Mitropoulos *et al.*, 2010; Huysmans and Longbottom, 2004; Kawato *et al.*, 2009). The damage experienced in this study for proximal surfaces was higher than that reported in previous studies where damage occurred to between 2% to 13% of specimens (Neuhaus *et al.*, 2015; Ástvaldsdóttir *et al.*, 2012; Jablonski-Momeni *et al.*, 2011). This slightly higher damage rate could be attributed to the age of the teeth as these were extracted and stored in tubs filled with tap water prior to 2006.

Qualitative studies including Micro-CT as validation method for occlusal and proximal caries detection					
AUTHOR	**Number of teeth & Type of teeth**	**Number of examiners**	**Diagnostic&Validation methods**	**Results**	**Comments**
(Matsuda *et al.*, 2002)	30 Premolar teeth on proximal surfaces. Caries prevalence in sample is 42%	Three Radiologists	Four combination modes of RVG UI system and intraoral film. Validation Micro-CT only.	All modes are viable alternative to detect early proximal lesions. Micro-CT is an acceptable validation method.	No comments
(Tsuchida *et al.*, 2007)	50 premolars on proximal surfaces. Caries prevalence at D_1 and D_3 threshold is 71%&24% respectively	One Radiologist	CBCT and conventional film. Validation Micro-CT only	3D Accuitomo Cone beam system is more accurate than convention film for caries detection. Micro-CT is a good validation method.	Micro-CT is a reliable method to determine the decalcification of dental hard tissues
(Kawato *et al.*, 2009)	27 Molar teeth (78 occlusal sites). Caries prevalence at D_1&D_3 threshold is 93.5% and 18% respectively	One dental practitioner	DIAGNOdent device, six teeth were serially sectioned in 100 microns thickness for histological validation	Micro-CT is alternative method for validation of DIAGNOdent device	Downer classification system was used for determining lesion depth

(Young *et al.*, 2009)	146 teeth with some cavitation (92 occlusal sites& 100 proximal surfaces). Caries prevalence of occlusal caries at D_1&D_3 threshold is 50% and 39% respectively	Two dental practitioner	CBCT, conventional CCD images, validation by Micro-CT (Scanco 40)	CBCT improved detection of caries lesions extended to dentine on proximal surfaces only	Caries prevalence of proximal caries at D_1&D_3 threshold is 50% and 25% respectively. Downer classification for determining lesion depth
(Mitropoulos *et al.*, 2010)	20 Premolars on proximal surfaces. Caries prevalence at D_1 &D_3 threshold is 60% and 45% respectively	Two dental practitioner	ICDAS II, digital BW and Micro-CT. Histology for validation.	R_s for Micro-CT and histology 0.77. Weighted kappa for Micro-CT 0.81. Accuracy of Micro-CT at D_1 and D_3 threshold 0.85 and 0.82 respectively	Teeth were hemi-sectioned. Micro-CT was not good for detecting early enamel lesions
(Kamburoglu *et al.*, 2011)	21 Molar teeth on occlusal surfaces. All teeth are affected by dentine lesions	Three Radiologists	Conventional film, CCD films, two types of CBCT and Micro-CT. Serial sections for validation	Lesion depth measurements are comparable for occlusal caries. Micro-CT can be used as non-destructive *in vitro* imaging modality.	Depth of lesion in millimetres (quantitative study)
(Soviero *et al.*, 2012)	24 Primary molar teeth with some cavities on proximal	Two dental practitioner	ICDAS II, BW & Micro-CT. Serial sections for validation	R_s for Micro-CT and histology 0.86. Accuracy of Micro-CT	Weighted kappa for Micro-CT and histology 0.87 and 0.95

	surfaces. Caries prevalence of proximal caries at D_1&D_3 threshold is 81% & 33% respectively			at D_1&D_3 threshold 0.88 and 0.98 respectively	respectively. Micro-CT is an alternative method for caries validation
(Dos Santos *et al.*, 2015)	11 Molars with hidden caries	Three dental practitioner	Visual and radiographic validation. Micro-CT for validation	Unweighted kappa (inter-examiner agreement) for Micro-CT 0.92	Micro-CT was able to detect communication of hidden lesions with oral cavity

Table 3.7 Summary of the relevant Micro-CT studies used for validation of caries detection methods using different classification criteria for lesion depth on occlusal and proximal surfaces.

As such they may be more brittle and prone to fracture during sectioning despite the fact that the thickness of each section was altered in an attempt to overcome this problem. Where fracture occurred on the proximal surfaces only the enamel was lost, regardless of the extension of the lesion into dentine (if present), allowing validation of dentinal lesions and calculation of sensitivity and sensitivity at the D_3 diagnostic threshold (see Chapter 4). To calculate the accuracy at the D_1 diagnostic threshold on the proximal surface where damage had occurred the consensus data from the preliminary inspection of the hand held teeth viewed under ideal conditions was used where any white opacities in enamel were classified as carious. In contrast, all the tomographic images were able to identify caries presence and extent based on the changes in the grey scale level (Davis *et al.*, 2013; Taylor *et al.*, 2010).

The weighted and unweighted kappa values, using Downer and ERK classification system, showed almost perfect agreement within and between the two examiners for both histology and Micro-CT, with the exception of a moderate agreement between both examiners for occlusal caries detection on tomographic images using the ERK classification system. The latter may be due to the more complex anatomy of the tooth in pits and fissures and the fact that the ERK classification system does not use the EDJ as a threshold and is therefore slightly more subjective.

The Micro-CT and histology weighted kappa values observed in this study for intra- and inter-examiner reproducibility are in accordance with those found in previous studies using a modified ERK (Soviero *et al.*, 2012) and modified Downer classification system (Mitropoulos *et al.*, 2010) (see Table 3.7). In other studies in which only histology has been used as the "gold standard" the reported inter-examiner kappa values for the whole classification system have been slightly lower in the order of 0.74 and 0.75 (Jablonski-

Momeni *et al.*, 2008; Jablonski-Momeni *et al.*, 2009) whist the intra-examiner reproducibility has been consistent with that in this study; kappa values between 0.83 and 0.76 (Jablonski-Momeni *et al.*, 2009) and between 0.81 and 0.93 (Cayley and Holt, 1997). In this study both weighted and unweighted kappa values have been calculated whereas in other studies one or the other only have been used, and in some studies it is unclear as to which test was used.

For both the Downer and ERK classification system there was a strong direct relationship between Micro-CT and histology consensus scores for both occlusal and proximal caries. However, this does not mean that both histology and Micro-CT are in perfect agreement when using both clinical classification systems as the percentage agreement ranged between 61% for occlusal caries and 88% for proximal caries. The difference in percentage agreement between the surfaces could be explained by the higher number of sound sites on the proximal surfaces compared to the occlusal and the more complicated anatomy of the latter. Comparable results have been observed also in previous studies demonstrating a strong relationship between Micro-CT and histology for proximal caries detection in primary and permanent teeth (Mitropoulos *et al.*, 2010; Soviero *et al.*, 2012).

Considering the cross-tabulation of consensus scores, for Micro-CT and histology, with respect to occlusal caries, and using both classification systems (Figure 3.4), the number of sound sites scored with Micro-CT is obviously higher than the number of sound sites determined from histology. The explanation may be related either to the histological examination, leading to false positives (hence lower number of sound sites) where staining or shadows lead to misinterpretation of sound sites or, due to the fact that, with Micro-CT, the degree of demineralisation was insufficient for lesion detection; the more demineralised the enamel, the easier it is to see on the Micro-CT image (Cochrane *et al.*,

2012). However, it has been shown that mineral changes can be detected even if limited to 100 microns depth (Lo *et al.*, 2010). It is possible that combining the subjective visual examination of the Micro-CT images with analysis of mineral density within the occlusal enamel lesion (if present) may improve the validation technique and make it a more objective and robust. This will be explored in more detail in Chapter 5. This issue does not arise on the proximal surfaces where tooth anatomy and lesion presentation is less complex.

Approximately nine occlusal dentinal lesions detected by Micro-CT, scored 3 with the Downer system, had been scored as enamel-only lesions using histology. This disagreement may very well highlight the problem that sectioning may abrade a considerable amount of dental tissue eliminated the deepest aspect of a lesion.

The performance of Micro-CT when validated by histology at the D_1 and D_3 diagnostic thresholds using the Downer classification system demonstrated a high degree of accuracy compared with histology. Up to now, only two known studies have reported these values (Table 3.7). Both studies investigated proximal caries, one for permanent teeth (Mitropoulos *et al.*, 2010) and one for primary teeth (Soviero *et al.*, 2012). In these studies, the diagnostic accuracy of Micro-CT was in agreement with that found in this study: Accuracy at D_1 Diagnostic threshold, Primary teeth = 0.88, permanent teeth =0.85, this study 0.94; accuracy at D_3 diagnostic threshold, Primary teeth = 0.98, permanent teeth = 0.82, this study 0.99.

It is almost impossible to achieve one hundred percent agreement between two different diagnostic or validation methods or to perform exactly in the same way under standard conditions on different occasions. The most useful approach is to evaluate the degree of the disagreement and whether the difference is large enough to cause problems in clinical

practice (Huysmans and Longbottom, 2004) or through the interpretation of research results (Martin Bland and Altman, 1986). These issues will be discussed more in detail in Chapter 4.

3.6 Conclusions

Within the limitations of this study, the conclusions that can be drawn from this Chapter are:

1. A precise and reliable locating technique was developed to accurately link occlusal investigation sites to histological section and corresponding Micro-CT image.
2. Fabrication of custom formed system based on three dimensional prototyping to accommodate the specimen allowed Micro-CT images to be obtained in the correct plane for comparison with that of histological sections.
3. There was a strong agreement within and between examiners for occlusal and proximal caries identification using Downer and ERK classification systems for both Micro-CT and histology.
4. The correlation between Micro-CT and histology was very strong indicating a strong relationship for the identification of occlusal and proximal caries using Downer and ERK classification systems as a "gold standard".
5. The high diagnostic performance of Micro-CT achieved, when compared with histology as the most accurate and conventional gold standard at D_1 and D_3 diagnostic thresholds, would suggest that Micro-CT has the potential to be used as an alternative to conventional histology for occlusal and proximal caries detection.

CHAPTER FOUR

Diagnostic Accuracy of Conventional and Novel Caries Detection methods as Determined by Histology and Micro-CT: An *In vitro* Study

4.1 Introduction

Diagnostic tests are widely used in medical science for determining the presence or absence of a disease. In dentistry, they are widely used for caries detection. The detection of dental caries in the early stages has become the main focus of contemporary clinical practice (Tam and McComb, 2001; Thomas *et al.,* 2001), but in spite of this, the detection of early small and shallow lesions, especially on occlusal and proximal surfaces, is still difficult for dental professionals (Chong *et al.,* 2003; Weerheijm *et al.,* 1997). Moreover, the progression rate of lesions has changed and many carious lesions undermine the enamel without leading to a directly visible cavity (Marthaler *et al.,* 1996; Mejàre *et al.,* 2004). Currently in clinical practice caries detection is carried out primarily with a visual examination (Kidd *et al.,* 1993; Gimenez *et al.,* 2015a) and with the aid of a bitewing radiograph (Wenzel, 2004a). These traditional methods are based on the subjective interpretation of the carious status of the tooth.

Due to the difficulty in accurately detecting and assessing occlusal caries in particular, newer more novel detection techniques have been introduced over the last few decades, with the aim of improving traditional visual and radiographic inspection (Pereira *et al.,* 2001). One validated visual system which fulfils this requirement is the "International Caries Detection and Assessment System" (ICDAS-II) (Ismail *et al.,* 2007). Other subjective methods are also available to aid detection of caries such as Fiberoptic Transillumination (Côrtes *et al.,* 2000), Panoramic Radiographs (DPT) (Thomas *et al.,* 2001) and Cone Beam-CT (Haiter-Neto *et al.,* 2008), which has the potential to be a valuable tool in dentistry by producing three dimensional images of the teeth and related structures. Other diagnostic devices have been developed and are commercially available which provide objective measurements of caries severity, for example the DIAGNOdent

Pen and the relatively newer device the CarieScan PRO. The former based on laser fluorescence and the latter on Electrical Impedance Spectroscopy (Gimenez *et al.*, 2013b; Mortensen *et al.*, 2014).

An ideal diagnostic device or technique should have a high sensitivity for both enamel and dentine lesions in order that early lesions can be treated preventively and before substantial tissue loss occurs, and have a high specificity to ensure sound surfaces are not unnecessarily treated operatively. Commonly, the validity of a detection system is established by using a gold standard against which the sensitivity (true positive proportion) and specificity (true negative proportion) can be calculated. Both traditionally used histology and the newer Micro-CT technology can be used for validation of different detection methods, using a precise and reliable locating system as described in Chapter 3.

This *in vitro* study therefore aimed to evaluate the diagnostic accuracy of conventional caries detection methods including ICDAS and digital bitewings; and novel caries detection methods including FOTI, Cone Beam-CT, DIAGNOdent Pen and CarieScan PRO using conventional histological serial sections and Micro-CT images for validation.

4.2 Materials and Methods

4.2.1 Study Sample:

The data from the eleven examiners using the conventional and novel detection methods in the examination of the 200 teeth described in Chapter 2 (244 occlusal sites (104 sites on premolar and 140 sites on molar teeth) and 400 proximal surfaces) was used in this Chapter to assess the diagnostic accuracy of the techniques. Consensus data from the histological examination allowed validation of all 200 teeth, both on the occlusal and

proximal surfaces was used in this study. Only 140 teeth underwent Micro-CT examination, therefore the diagnostic accuracy of the detection methods were determined on these 140 teeth using both histology and Micro-CT validation to allow direct comparison.

4.2.2 Conventional Caries Detection Methods:

This part of the study includes the visual examination (ICDAS) and digital bitewing radiographs for the detection of occlusal and proximal caries.

The occlusal investigation sites were marked with a ring on a black and white print out of the occlusal surface to ensure all examiners examined the same sites; where multiple occlusal investigation sites were chosen the apparently worst affected site was always included.

Visual (ICDAS) Examination: For the ICDAS visual examination (see Chapter 2) the teeth were set up in arches and placed in phantom heads. The teeth were viewed wet and dry, under a standard dental operating light, using a new disposable mirror and plastic WHO probe to confirm any surface discontinuities.

Digital Bitewing Radiograph: Bitewing radiographs of teeth were taken with the maxillary and mandibular arches mounted on an articulator using no soft tissue equivalent. All radiographs were viewed by all examiners under the same dimmed ambient lighting (see Chapter 2).

4.2.3 Novel Caries Detection Methods:

AC Impedance Spectroscopy (CarieScan PRO): readings were carried out first on the occlusal surfaces only, before setting the teeth in arches, whereas for all other novel

detection methods such as FOTI, DIAGNOdent pen and Cone beam-CT were carried out with the teeth set up in the arches to simulate the clinical situation as precisely as possible.

FOTI and DIAGNOdent pen examinations were carried out on dried teeth in the phantom head, for the FOTI examinations the operating light was switched off.

Cone Beam CT radiographs were taken with the arches held together with an elastic band with no soft tissue equivalent, using i-CAT®Imaging Cone Beam next generation CT system. Each examiner viewed the **serial Cone Beam CT images** of each tooth in the sagittal (mesio-distal) plane under standardised ambient conditions as for all radiographic examinations. For each pair of arches an **ideal panoramic 2D image** of 0.125 mm slice thickness was also created for examination (see Chapter 2).

Due to the large number of unreadable (56.5%) occlusal and (66%) proximal tooth surfaces on the DPT, further analysis of the DPT results was not undertaken.

4.2.4 Micro-CT Validation Technique:

A total of 140 out of the entire 200 teeth used in Chapter 2 were scanned using a small-angle-cone-beam desktop μCT 40 Scanco system. For the occlusal surface each investigation site was classified according to two classification systems, namely the Downer (Table 3.1) and ERK (Table 3.2) systems, by two examiners and consensus score reached (Chapter 3). When checking the Micro-CT images (slices) if the deepest aspect of the lesion (if present) was not at an investigation site, then this additional deepest aspect of the lesion was also recorded. For each proximal surface, caries status was also classified according to the Downer and ERK classification systems and the deepest aspect of the lesion was recorded.

The inter- and intra-examiner reproducibility of the Micro-CT examinations of the 140 teeth (50% for intra-examiner reproducibility) that underwent Micro-CT was presented in Chapter 3.

4.2.5 Conventional Histological Validation Technique:

The entire sample of 200 embedded teeth were serially sectioned in a mesio-distal direction. The photographic image of the histological section corresponding to each occlusal investigation site was recorded for classification by two examiners according to the two subjective classification systems presented in Table 3.1 and 3.2 and used for the Micro-CT images. Where disagreement between examiners occurred consensus was reached by re-examination and discussion and the consensus score was used as the so called "site specific gold standard". This was used to validate site specific detection methods such as ICDAS, FOTI, DIAGNOdent pen and CarieScan Pro. Where the depth of the lesion (if present) beneath the investigation sites did not correspond to the deepest aspect of the lesion, the image of the section with this new deepest lesion was also selected for classification. The consensus score at this deepest lesion site was used as the "overall gold standard" for the occlusal surface. This "overall gold standard" was used to validate surface specific detection methods namely the radiographic techniques (digital bitewing radiographs and cone beam CT images).

For proximal surfaces the image of the section with the deepest aspect of the lesion (if present) was also selected for classification using the same classification systems. The consensus score was used as the "proximal surface gold standard".

For each examiner 50% of the investigation sites (50% occlusal and 50% proximal) were randomly selected for re-examination.

4.2.6 Statistical Analysis:

Reproducibility and distribution of histological scores for 200 teeth: The weighted kappa statistic (Landis and Koch, 1977) was used to evaluate inter- and intra-examiner agreement for histological examination for occlusal and proximal surfaces using each classification system for all 200 teeth. All weighted kappa analyses were carried out using MedCalc V 13.1.2.0 statistical software (MedCalc Software bvba, Belgium), and the level of significance was set at $P < 0.05$. Cohen's unweighted kappa was also applied to assess inter- and intra-examiner reproducibility for histological examination using SPSS software V 21(PASW statistics, SPSS Inc., Chicago, USA).

Descriptive statistics (frequencies) were used to summarize the frequency distribution of consensus scores given by conventional histological examination for occlusal and proximal surface examination of 200 teeth, using the Downer and ERK classification systems, using SPSS software V 21(PASW statistics, SPSS Inc., Chicago, USA) and Excel 2013 (Microsoft Corporation, USA).

Relationship between Micro-CT, histology and each detection method: The Spearman correlation coefficient (r_s) was calculated to examine the relationship between consensus Micro-CT scores (using the Downer Criteria only) and the results from each detection method (for 140 teeth). For the DIAGNOdent Pen and the CarieScan PRO the actual readings were used, not the converted categorical data described in Chapter 2. This was done separately for occlusal and proximal surfaces using SPSS software V 21(PASW statistics, SPSS Inc., Chicago, USA). The Micro-CT score at each investigation site was used for site specific examinations (ICDAS, FOTI, DIAGNOdent, CarieScan) (n=172 sites) and the deepest site per tooth was used for the radiographic techniques. This was also carried out to explore the relationship between histology and each detection method

separately for occlusal and proximal surfaces in a similar manner for the entire sample 200 teeth (excluding investigation sites of damaged sections).

The percentage agreement between the Micro-CT and histology consensus scores and the results from each detection method, for each examiner, on each tooth surface were also calculated for 140 and 200 teeth respectively in a similar manner as aforementioned. The scoring systems for the ICDAS, radiographic techniques and FOTI all relate directly to the Downer classification system therefore calculation of the percentage agreement between these detection methods and histology is logical. However, with the continuous data and previously published thresholds determined by the manufacturer or in research papers, means that direct comparison between DIAGNOdent and CarieScan readings and histology was less appropriate and the percentage agreement for these techniques was not determined.

Diagnostic accuracy for each detection method validated by both Micro-CT and histology (140 teeth): Using the consensus histology and Micro-CT results for the Downer histological classification system as the "gold standard" to validate the caries detection methods for occlusal and proximal caries, sensitivity (Se) and specificity (Sp) were calculated at the D_1 diagnostic threshold (any lesion in enamel or dentine classed as caries; Downer cut-off between classification 0/1) and at the D_3 diagnostic threshold (only lesions in dentine classed as caries; Downer cut-off between 2/3) for each examiner.

To calculate the sensitivity and specificity values, the data obtained by each examiner using each detection method was converted into dichotomous data by using the specific cut-off points for each examination technique, for the D_1 and D_3 diagnostic threshold as detailed in Table 2.7 (Chapter 2). Note two cut-off points have been used for ICDAS for

the D_3 diagnostic threshold as the ICDAS does not use the enamel dentine junction as a specific cut-off histologically. This was carried out first for the140 teeth validated by both Micro-CT and histology (for comparison) for both the occlusal (n=172 sites) and proximal surfaces (n=280 surfaces), including the investigation sites where damage had occurred during sectioning using the method described in Chapter 3 to achieve a "site specific gold standard". Note that the "site specific gold standard" was used for site specific readings for ICDAS, FOTI, DIAGNOdent and CarieScan and the "overall gold standard" (deepest aspect of the lesion if present) was used for the radiographic techniques.

In addition to this site specific validation for occlusal caries, for teeth where there were more than one occlusal investigation sites, the worse score or reading for the surface was also recorded for ICDAS, FOTI, DIAGNOdent Pen and CarieScan PRO examination technique for each examiner. The consensus histological and Micro-CT scores representing the deepest aspect of an occlusal lesion (if present) were used in this case to achieve the "overall gold standard" for validation of the most severe diagnostic score/reading. This also allowed more meaningful comparison with the radiographic detection methods at D_1 diagnostic threshold and D_3 diagnostic threshold.

Diagnostic accuracy for each detection method validated by histology for the whole sample (200 teeth): Using the consensus histology results for the Downer histological classification system for the 200 teeth as the "gold standard" to validate the caries detection methods for occlusal (n=244) and proximal caries (n=400), sensitivity (Se) and specificity (Sp) were calculated at the D_1 and D_3 diagnostic threshold for each examiner in a similar manner as described previously to achieve site specific and overall validation.

Inter-examiner variation was assessed by calculating the mean and standard deviation for sensitivity and specificity values obtained for each detection method at both diagnostic thresholds. The coefficient of variation was then calculated by dividing the standard deviation by mean and expressing the resultant proportion as a percentage. The higher the percentage the larger the variation between examiners (Everitt, 1998).

4.3 Results

A total of 200 teeth were examined with histology and only 140 teeth were examined by Micro-CT as discussed further in Chapter 3. The results for both techniques pertaining to these teeth will be considered further in this Chapter for the purpose of comparison and validation of conventional and novel caries detection methods.

Reproducibility and distribution of histological scores for 200 teeth: Regarding the histological examination of the whole sample (100 molar and 100 premolar teeth), in total there were 244 investigation sites on the occlusal surfaces and 400 proximal surfaces examined. During sectioning for histological examination 11 occlusal investigation sites (4.5%) and 60 proximal surfaces (15%) were lost due to section destruction. This left 233 occlusal investigation sites and 340 proximal surfaces for examination. On the occlusal surface one of the pre-selected examination sites represented the deepest aspect of the lesion for that tooth in 95% of cases. In 10 teeth the deepest aspect of the occlusal lesion was at a different site and this location was also recorded using the length of the composite triangle and was considered for further comparison only when determining the reproducibility of the histological technique making a total of 243 occlusal investigation sites and 340 proximal surfaces. Where section damage occurred this involved loss of

enamel only from the dentine and no dentine/lesion damage. A flow chart representing the procedure for histology is illustrated in Figure 4.1.

The inter- and intra-examiner weighted and unweighted kappa values for histological examination of 200 teeth for occlusal (243 occlusal investigation sites) and proximal (340 surfaces) caries using Downer and ERK scoring systems can be seen in Table 4.1.

The frequency distributions of both the Downer and ERK consensus scores for occlusal investigation sites and proximal surfaces determined from histological examination of 200 teeth is presented in Figure 4.2.

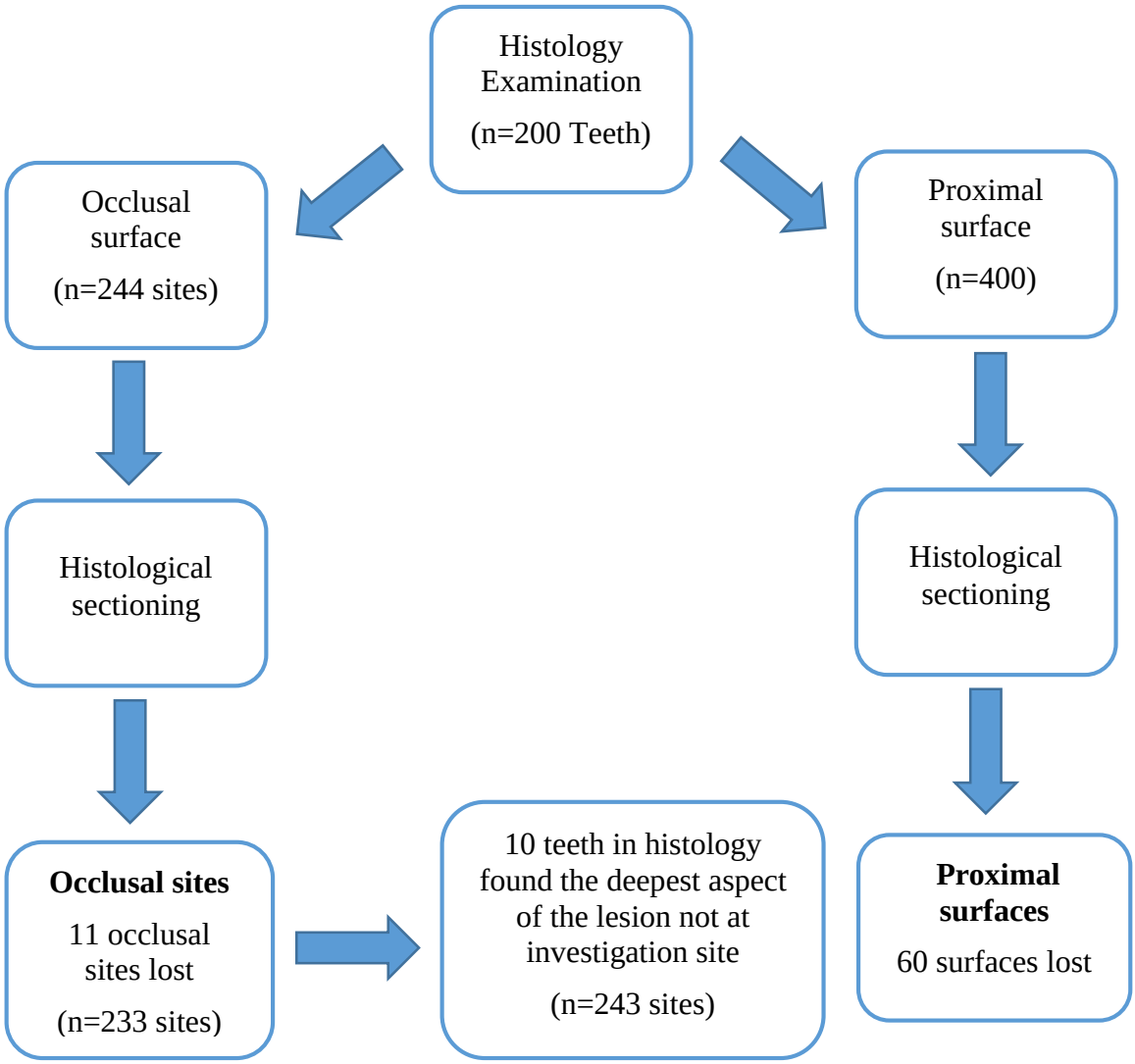
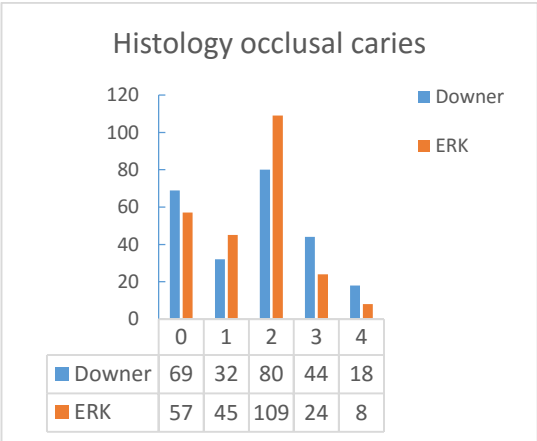


Figure 4.1 Flow chart representing the validation procedure using histology for the whole sample.

	Inter-examiner Reproducibility		Intra-examiner Reproducibility			
			Ex 1		Ex 2	
	Downer	ERK	Downer	ERK	Downer	ERK
Occlusal N=243	0.87 (0.73)	0.80 (0.72)	0.90 (0.84)	0.90 (0.85)	0.91 (0.85)	0.94 (0.86)
	0.95 (0.88)	0.95 (0.87)	0.94 (0.88)	0.91 (0.84)	0.94 (0.89)	0.95 (0.87)
Proximal N=340	0.97 (0.90)	0.97 (0.92)	0.95 (0.89)	0.98 (0.91)	0.96 (0.90)	0.98 (0.90)
	0.95 (0.89)	0.97 (0.91)	0.95 (0.90)	0.96 (0.86)	0.95 (0.88)	0.93 (0.86)

Table 4.1 Summary of inter- and intra- examiner weighted and unweighted kappa values for the histological examination using the Downer and the ERK scoring systems; unweighted kappa is in parenthesis; N is the number of occlusal investigation sites and proximal surfaces.

All the results for inter- and intra-examiner reproducibility indicated very strong agreement between and within examiners both for Downer and ERK classification systems.



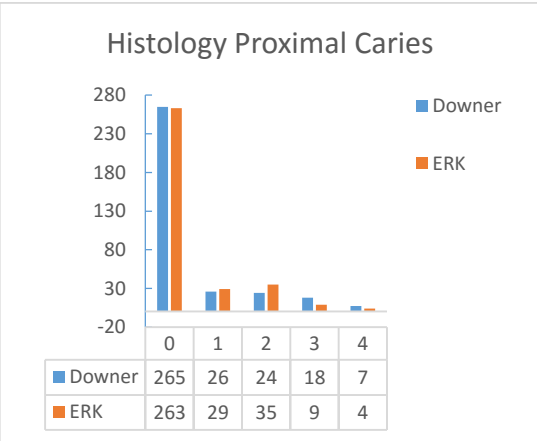


Figure 4.2 Frequency distribution of Downer and ERK histology consensus scores for 243 occlusal investigation sites and 340 proximal surfaces.

Based on the consensus Downer classification scores, the histological examination for the whole sample consisting of 200 occlusal surfaces; taking into account the deepest aspect of the lesion and 244 occlusal investigation sites; including the damaged sections after inspection for enamel or dentine caries as described in Chapter 3, revealed that only 56 occlusal surfaces (73 investigation sites were caries free), resulting in a caries prevalence at the D_1 diagnostic threshold of 72% for the surface and 70% at the investigation sites. Dealing with the damaged sections in the same way as in Chapter 3, for the 400 proximal surfaces, 313 surfaces were caries free, resulting in a caries prevalence of 22% at the D_1 diagnostic threshold.

At the D_3 diagnostic threshold the prevalence of occlusal caries was 25% for the surface and at the investigation sites (149 surfaces and 183 investigation sites were caries free). For the proximal surface the prevalence of caries at the D_3 threshold was 7% (373 surfaces were caries free).

Taking an example of each ICDAS consensus score for occlusal caries (determined by the three examiners in the preliminary inspection of the teeth prior to inclusion in this study), the corresponding digital bitewing image, and the corresponding Micro-CT image and histological section can be seen in Figures 4.3-4.7. Figures 4.8 – 4.12 also shows an example of each ICDAS consensus score (determined in the preliminary examination by direct vision) for proximal surfaces, together with the bitewing, CBCT and the corresponding Micro-CT and histological section.

An example of enamel and dentine lesions seen by FOTI and Cone Beam-CT slice in sagittal plane is illustrated in Figure 4.13 and 4.14 respectively.

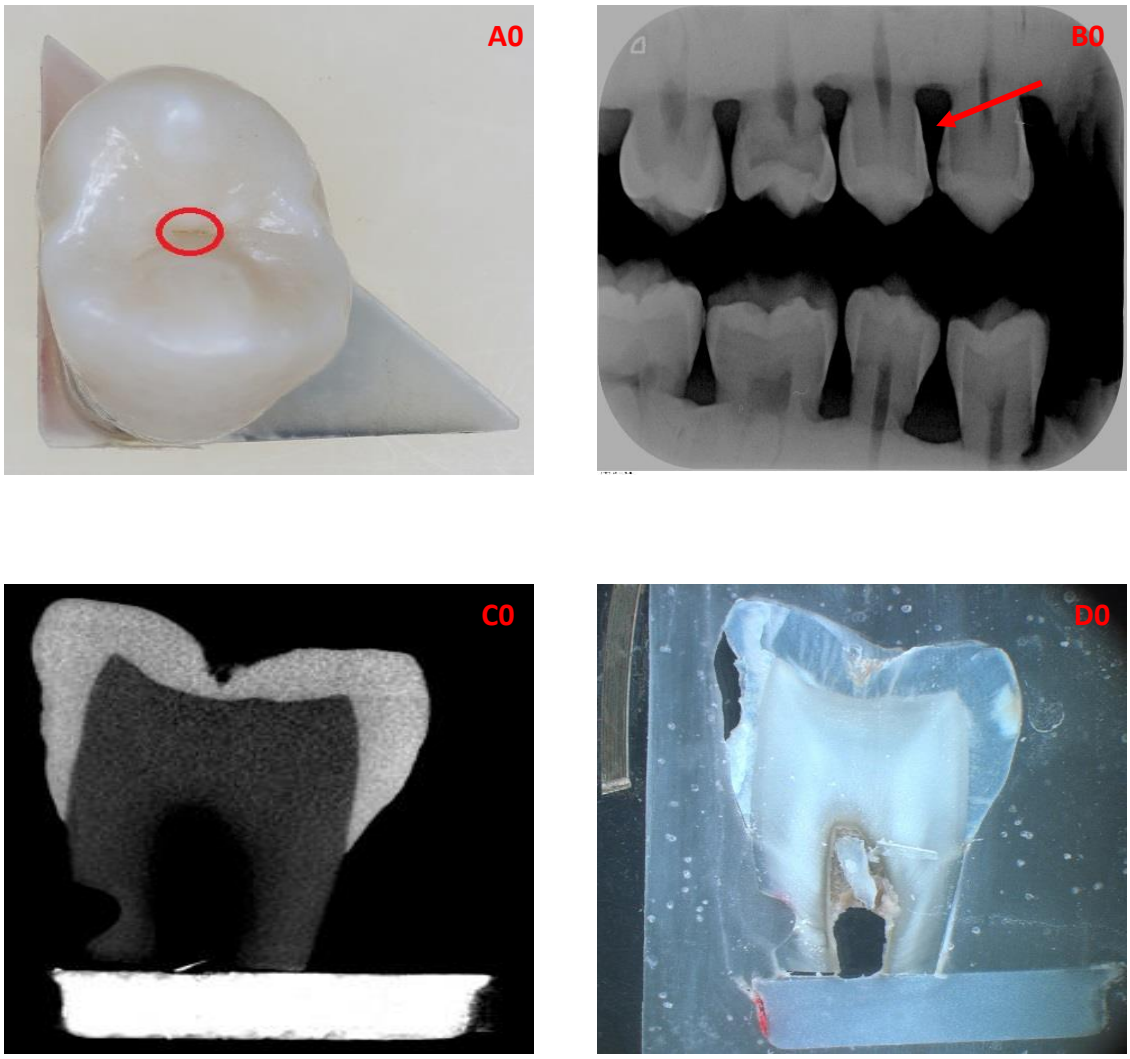


Figure 4.3 A group of images including a photograph of an occlusal surface of a tooth with 11x11x15.5 mm right angle isosceles triangle of composite bonded to its resected root together with a marked preselected investigation site scored using ICDAS classification system with **score 0** (A), the digital bitewing radiograph of the corresponding tooth (B), the Micro-CT image (C) and the histological section (D).

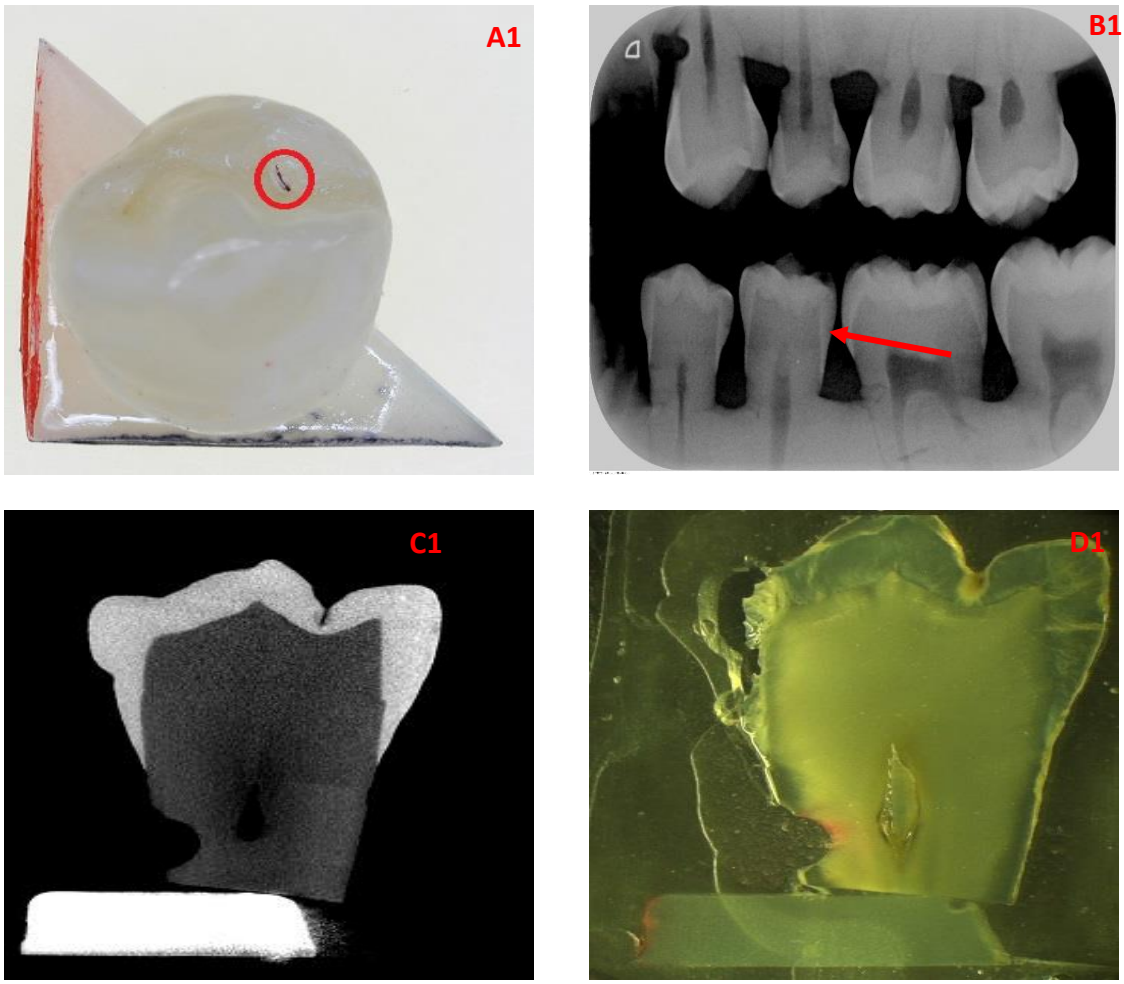


Figure 4.4 A group of images including a photograph of an occlusal surface of a tooth with 11x11x15.5 mm right angle isosceles triangle of composite bonded to its resected root together with a marked preselected investigation site scored using ICDAS classification system with **score 1** (A), the digital bitewing radiograph of the corresponding tooth (B), the Micro-CT image (C) and the histological section (D).

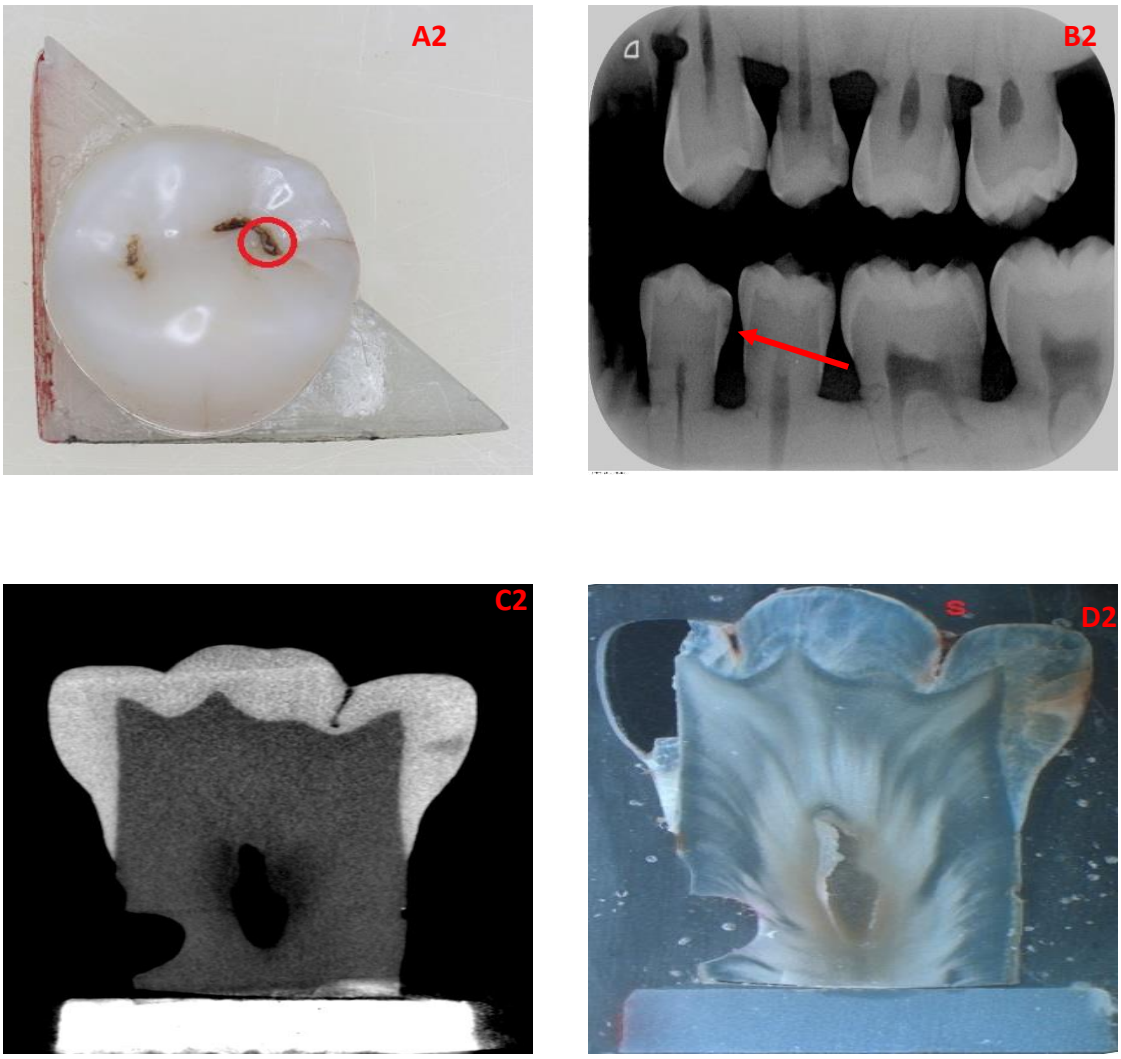


Figure 4.5 A group of images including a photograph of an occlusal surface of a tooth with 11x11x15.5 mm right angle isosceles triangle of composite bonded to its resected root together with a marked preselected investigation site scored using ICDAS classification system with **score 2** (A), the digital bitewing radiograph of the corresponding tooth (B), the Micro-CT image (C) and the histological section (D).

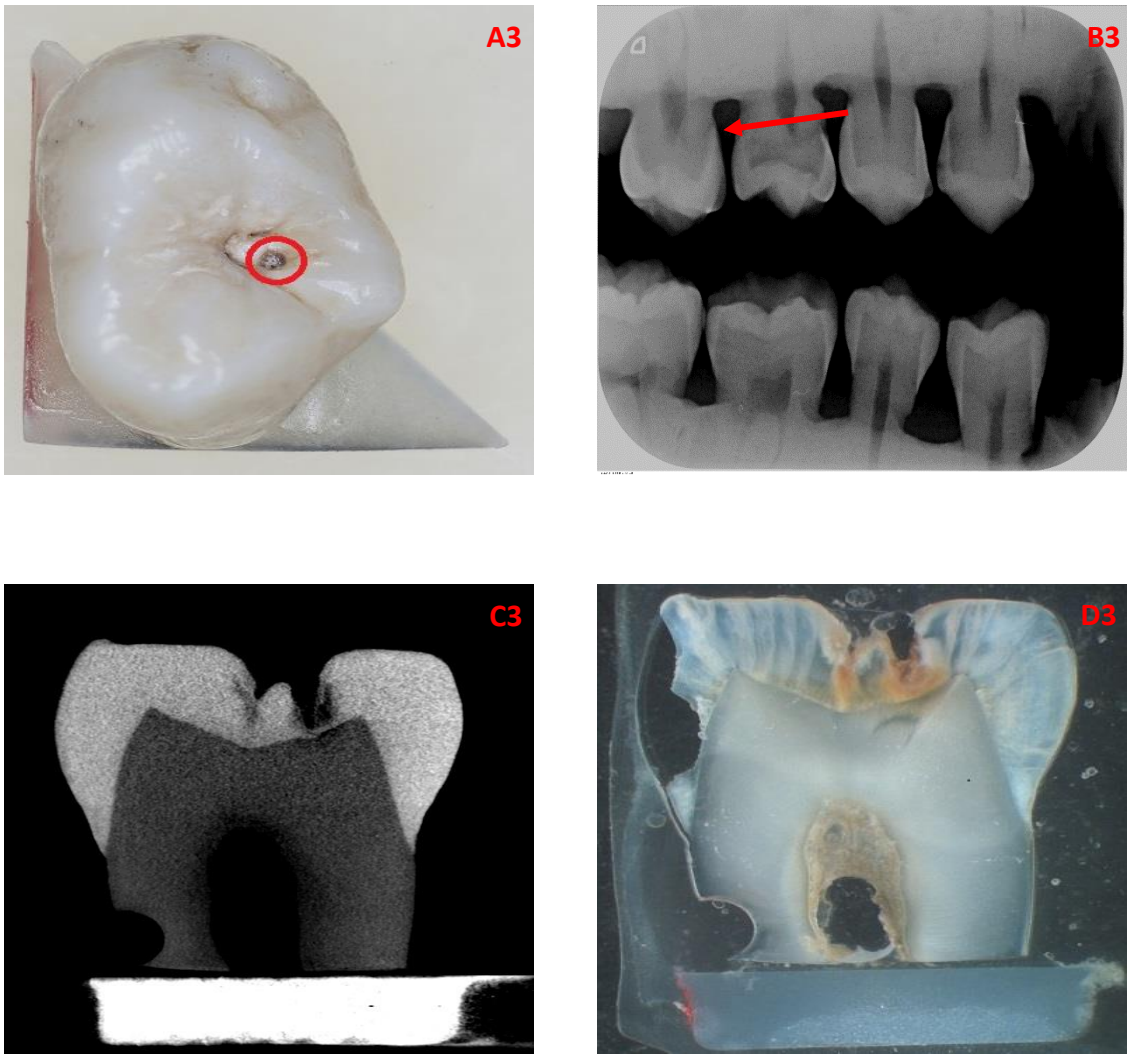


Figure 4.6 A group of images including a photograph of an occlusal surface of a tooth with 11x11x15.5 mm right angle isosceles triangle of composite bonded to its resected root together with a marked preselected investigation site scored using ICDAS classification system with **score 3** (A), the digital bitewing radiograph of the corresponding tooth (B), the Micro-CT image (C) and the histological section (D).

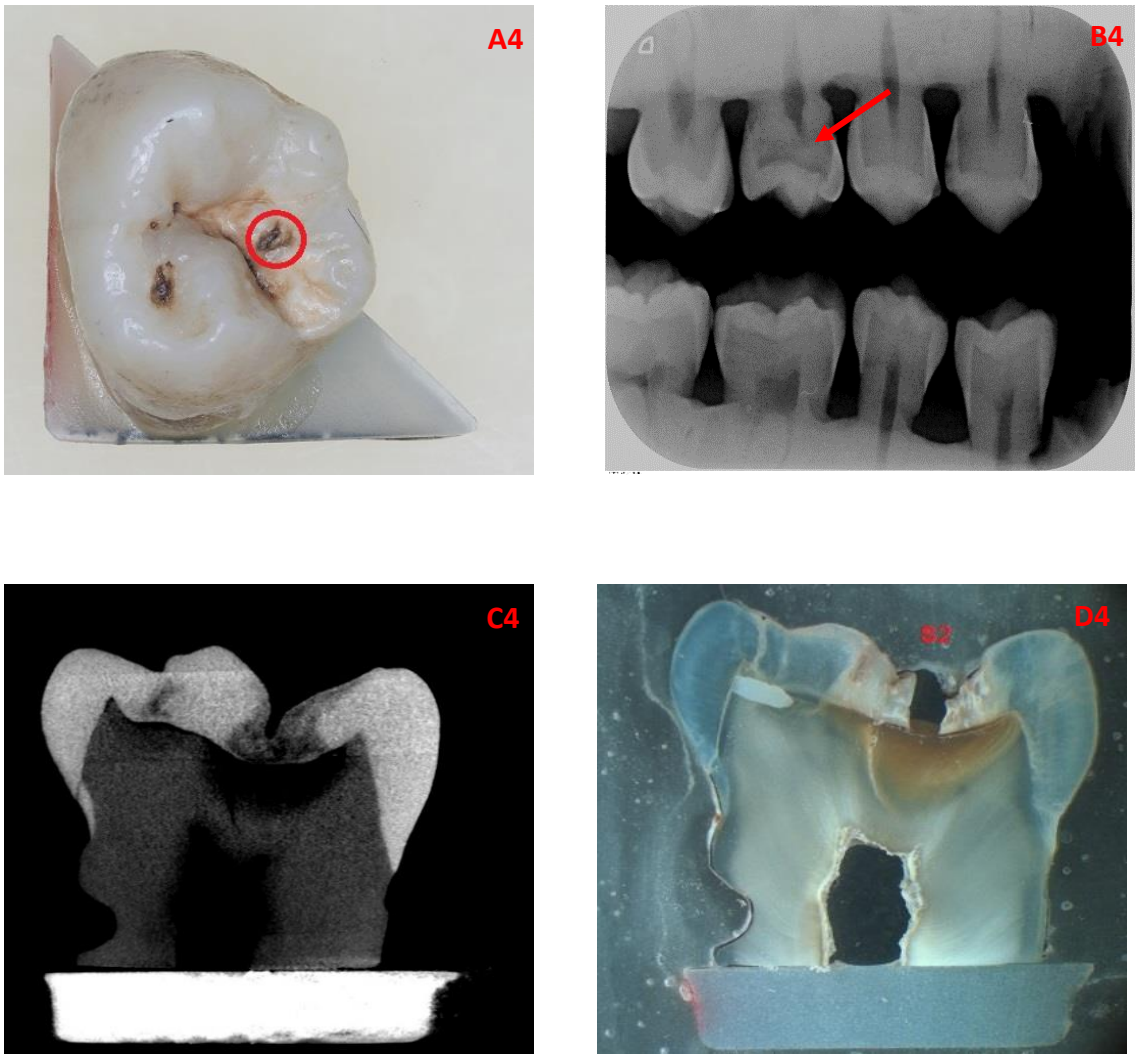


Figure 4.7 A group of images including a photograph of an occlusal surface of a tooth with 11x11x15.5 mm right angle isosceles triangle of composite bonded to its resected root together with a marked preselected investigation site scored using ICDAS classification system with **score 4** (A), the digital bitewing radiograph of the corresponding tooth (B), the Micro-CT image (C) and the histological section (D).

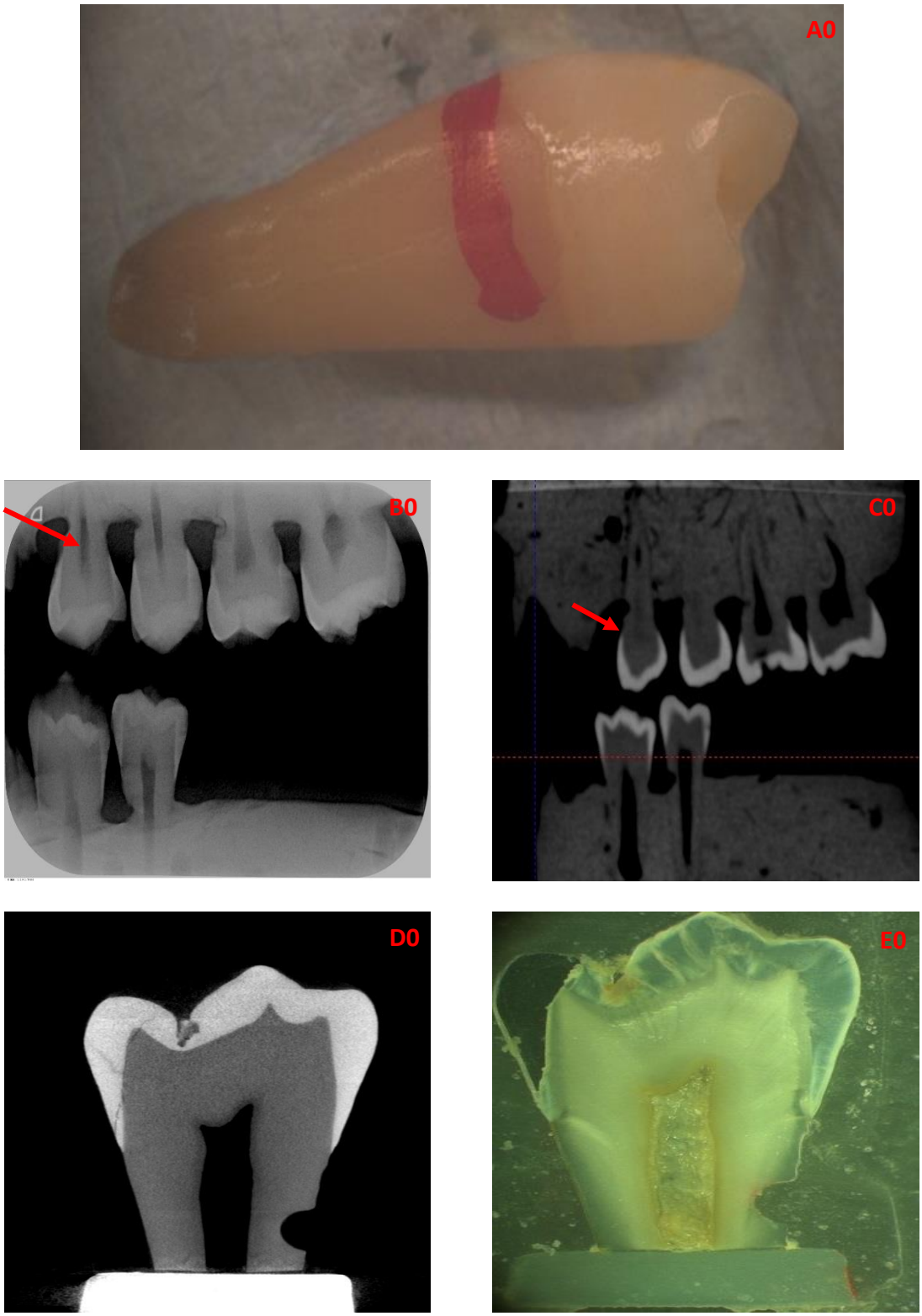


Figure 4.8 A group of images showing a photograph of the proximal surface under investigation scored using ICDAS classification system with **score 0** (A), the digital bitewing radiograph (B), CBCT image (C) of the corresponding tooth, the Micro-CT image (D) and the histological section (E).

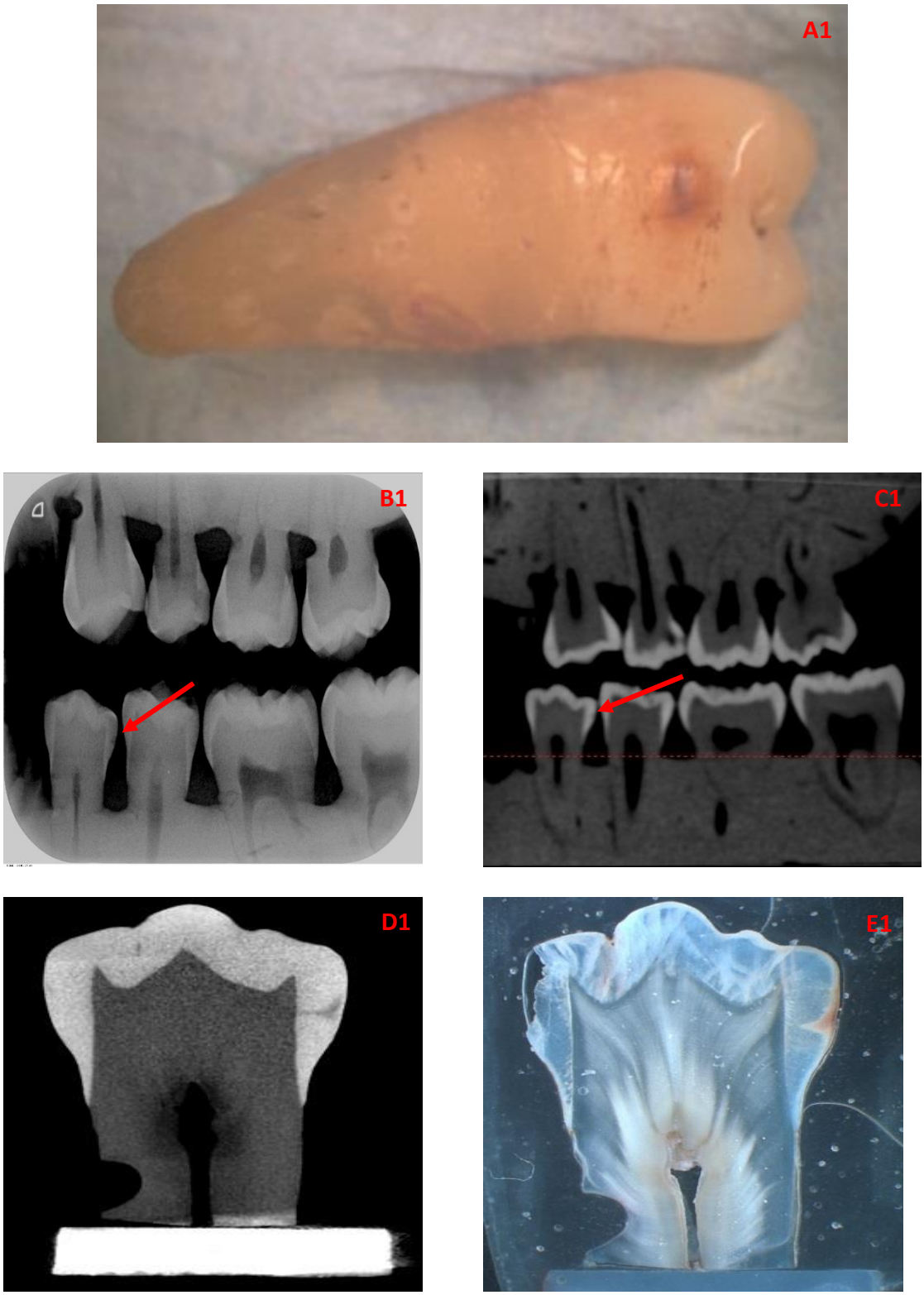


Figure 4.9 A group of images showing a photograph of the proximal surface under investigation scored using ICDAS classification system with **score 1** (A), the digital bitewing radiograph (B), CBCT image (C) of the corresponding tooth, the Micro-CT image (D) and the histological section (E).

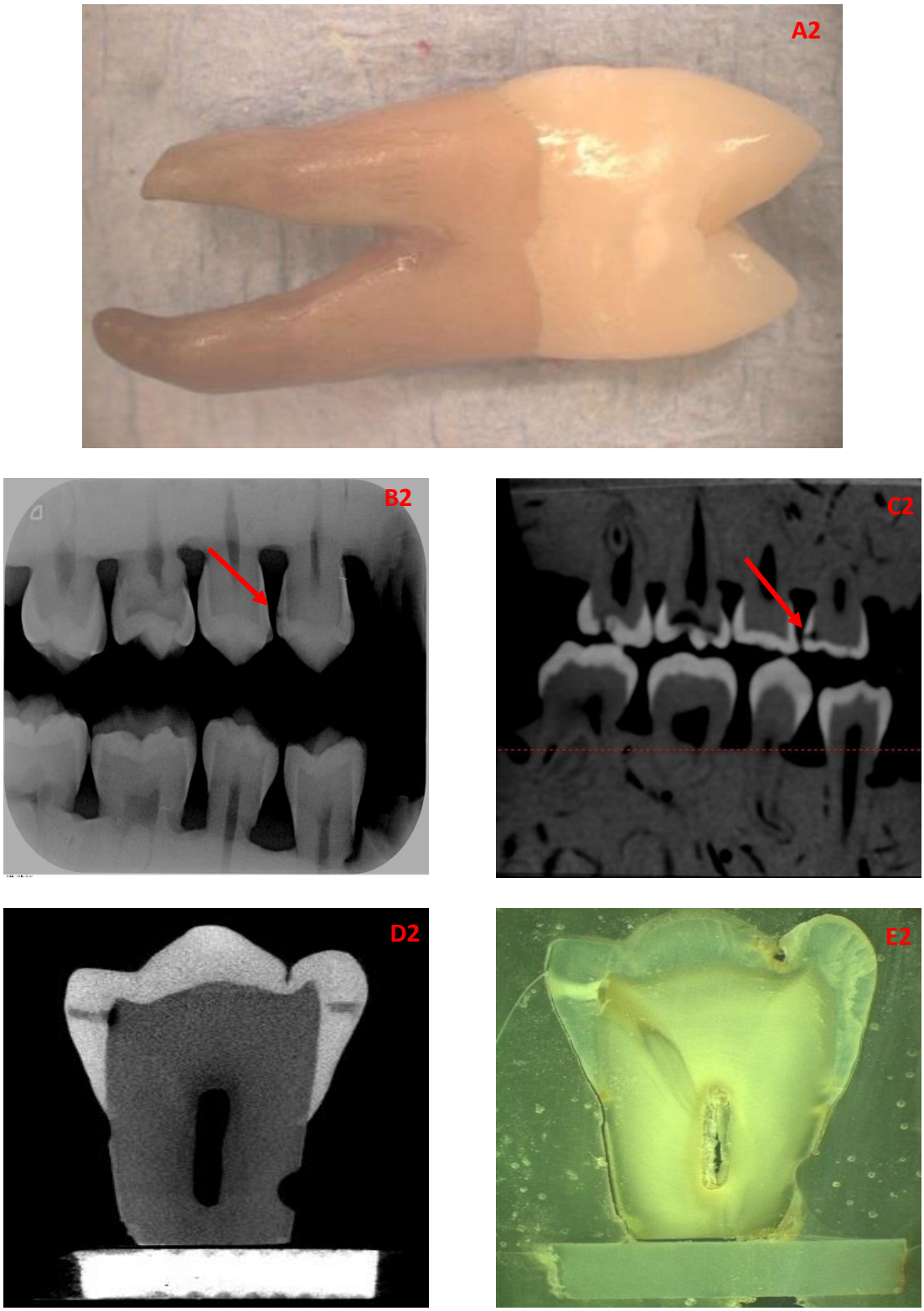


Figure 4.10 A group of images showing a photograph of the proximal surface under investigation scored using ICDAS classification system with **score 2** (A), the digital bitewing radiograph (B), CBCT image (C) of the corresponding tooth, the Micro-CT image (D) and the histological section (E).

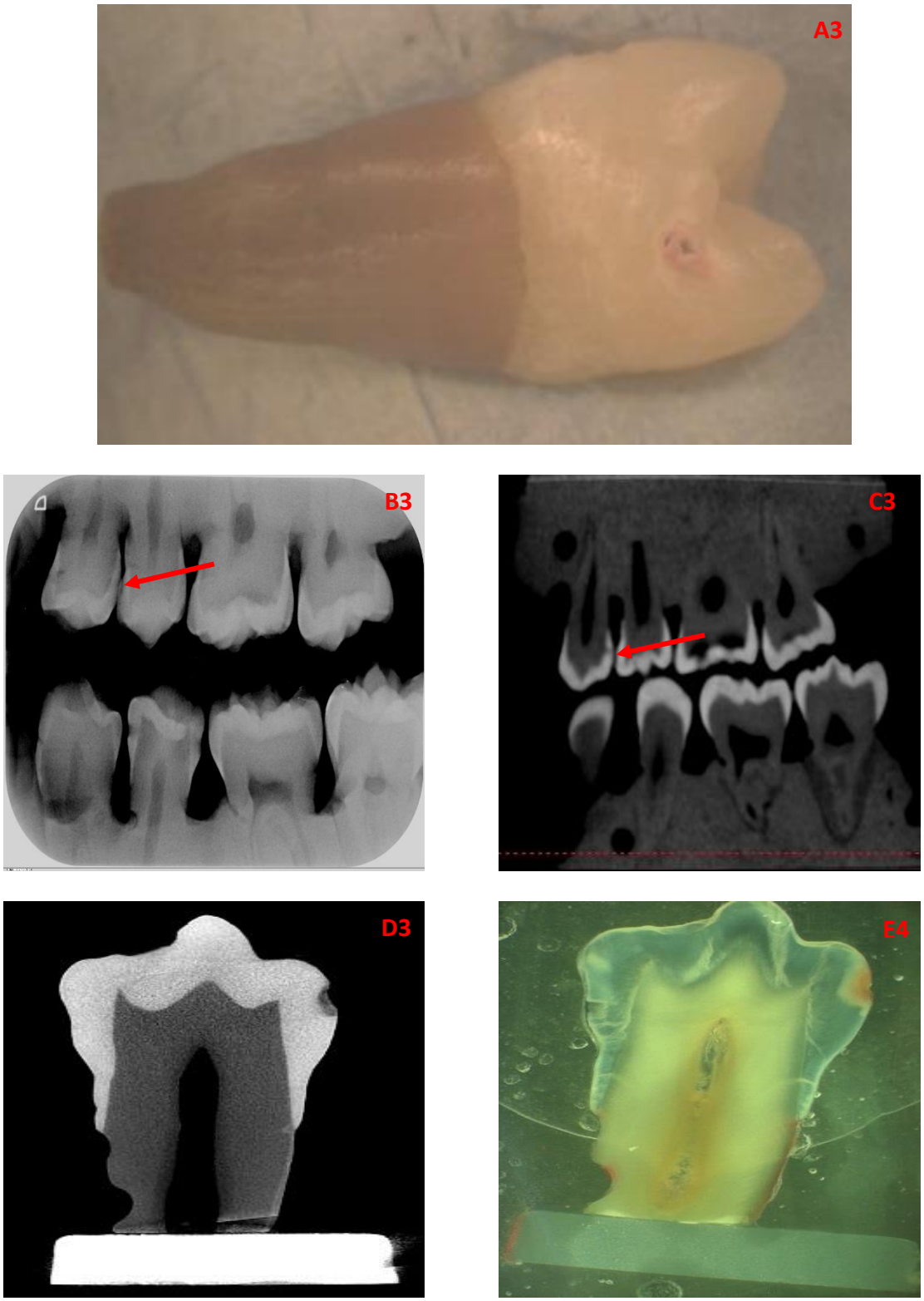


Figure 4.11 A group of images showing a photograph of the proximal surface under investigation scored using ICDAS classification system with **score 3** (A), the digital bitewing radiograph (B), CBCT image (C) of the corresponding tooth, the Micro-CT image (D) and the histological section (E).

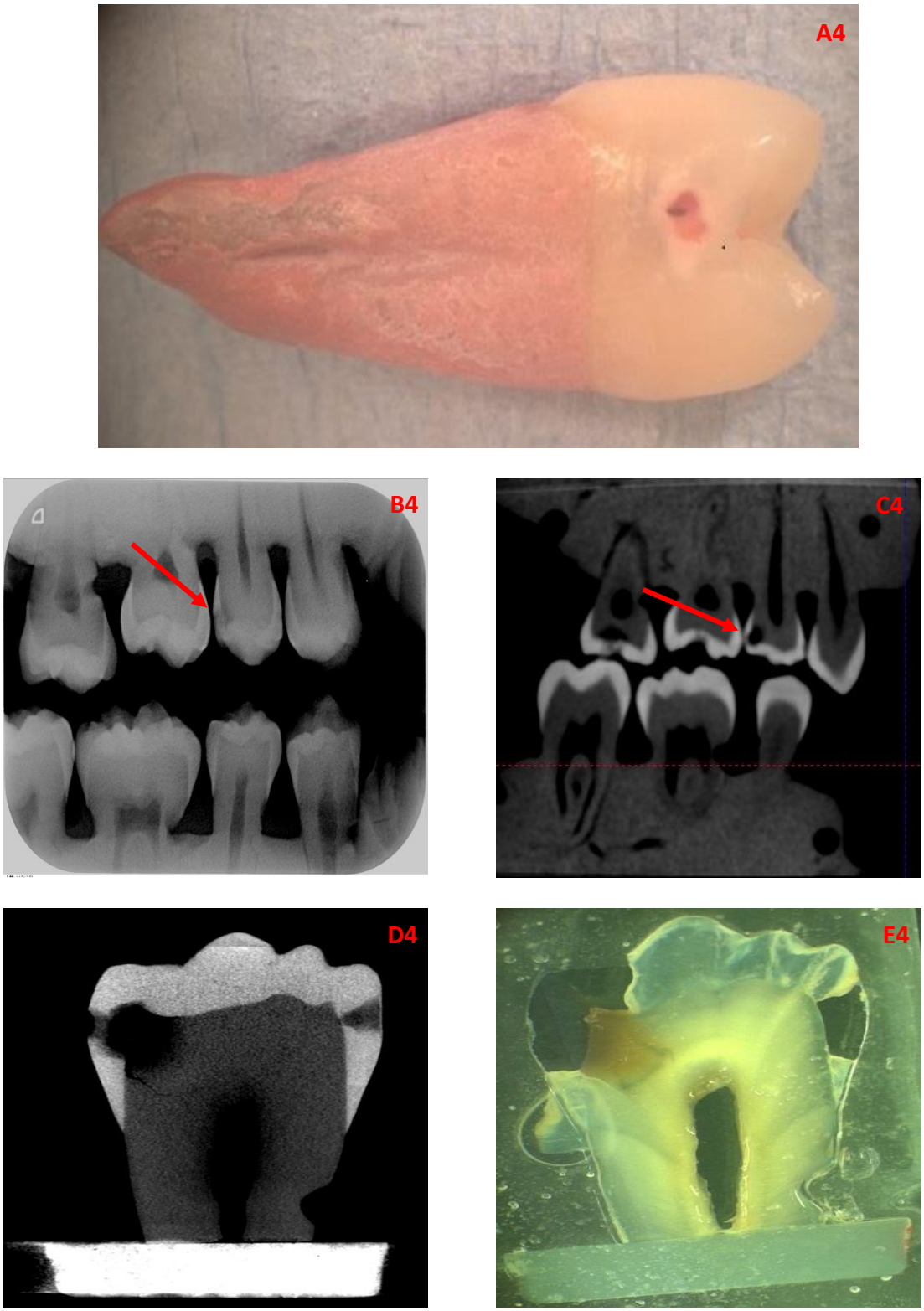


Figure 4.12 A group of images showing a photograph of the proximal surface under investigation scored using ICDAS classification system with **score 4** (A), the digital bitewing radiograph (B), CBCT image (C) of the corresponding tooth, the Micro-CT image (D) and the histological section (E).

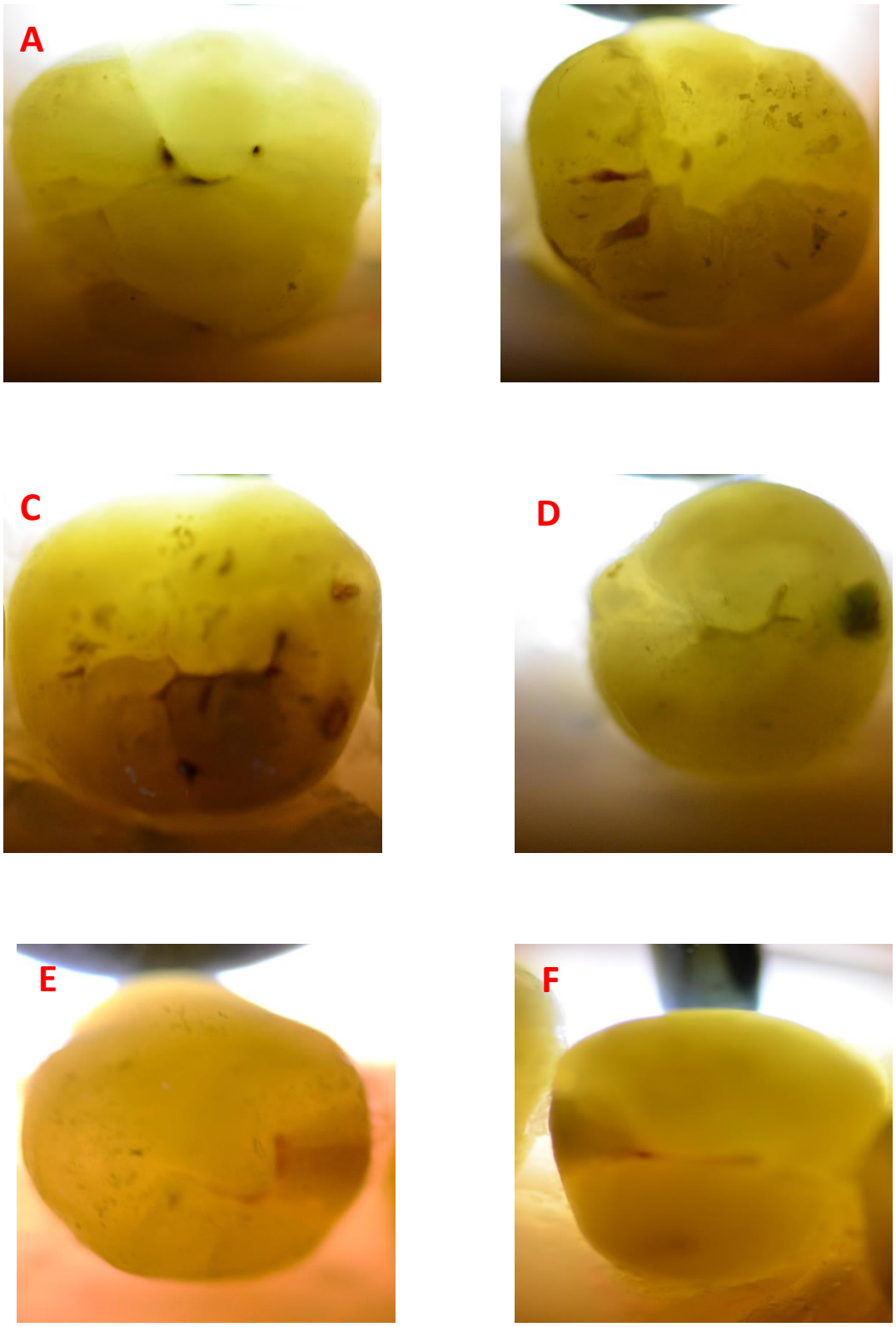


Figure 4.13 FOTI images of typical enamel and dentine lesions showing FOTI score 1 (A), score 2, score 4 (C) occlusal caries and, score 2 (D), score 3 (E), score 4 (F) proximal caries.

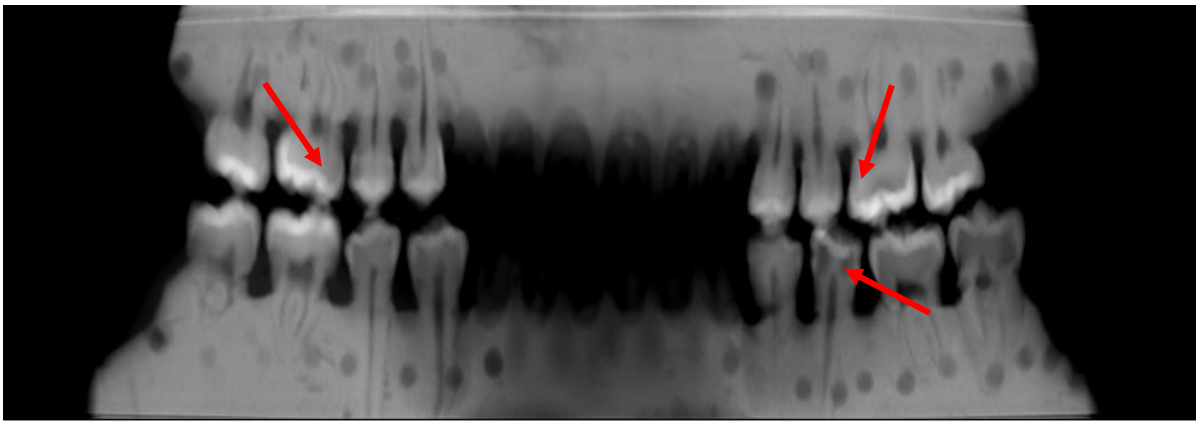


Figure 4.14 Cone beam-CT slice (panoramic 2D image) in sagittal plane showing typical occlusal and proximal lesions extending to dentine.

Relationship between Micro-CT, histology and each detection method: The relationship (mean Spearman's correlation coefficient (r_s) and mean percentage agreement) between consensus Micro-CT and histological examination (using the Downer classification) and the results obtained with each detection method, for occlusal and proximal caries, for all examiners can be seen in Table 4.2 (occlusal) and Table 4.3 (proximal).

The relationship (Spearman Correlation Coefficient) between histology, Micro-CT and caries diagnostic tests for occlusal caries detection was moderate except for Bitewing radiographs and CarieScan PRO which had a weak relationship. However, for proximal caries the relationship was weaker for DIAGNOdent Pen and Panoramic view of CBCT and was not significant for digital Bitewing radiograph.

The Percentage agreement between the histology and Micro-CT consensus scores for proximal caries, using Downer classification systems with all other caries diagnostic tests was higher than that for occlusal caries.

	Histology N=200		Micro-CT N= 140	
	r_s (SD)	% Agreement (SD)	r_s (SD)	% Agreement (SD)
ICDAS	0.61* (0.06)	59% (5.1)	0.62* (0.04)	57% (6.1)
CBCT	0.63* (0.06)	48% (3.3)	0.65* (0.05)	49% (4.1)
FOTI	0.59* (0.05)	53% (6.2)	0.61* (0.04)	56% (5.9)
DIAGNOdent Pen	0.63* (0.01)	NA	0.62* (0.01)	NA
Panoramic view of CBCT	0.41* (0.07)	38% (6.8)	0.42* (0.08)	37% (7.1)
Bitewing Radiographs	0.32* (0.09)	33% (5.3)	0.35* (0.1)	36% (5.2)
CarieScan PRO	0.21* (0.1)	NA	0.23* (0.09)	NA

Table 4.2 The mean (SD) Spearman's correlation coefficient (r_s) and the mean percentage agreement (SD) for occlusal caries detection between histology or Micro-CT and all diagnostic tests. * denotes P < 0.001. NA = Not Applicable

	Histology N=400		Micro-CT N= 280	
	r_s (SD)	% Agreement (SD)	r_s (SD)	% Agreement (SD)
CBCT	0.58* (0.03)	73% (3.9)	0.59* (0.05)	74% (4.1)
ICDAS	0.57* (0.04)	71% (4.1)	0.59* (0.04)	73% (3.8)
FOTI	0.52* (0.06)	69% (5.6)	0.53* (0.07)	67% (5.8)
DIAGNOdent Pen	0.32* (0.01)	NA	0.34* (0.0)	NA
Panoramic view of CBCT	0.23**(0.06)	52% (8.1)	0.25**(0.05)	54% (8.3)
Bitewing Radiographs	0.06 NS	45%	0.09 NS	46%

Table 4.3 The mean (SD) Spearman's correlation coefficient (r_s) and the mean percentage agreement (SD) for proximal caries detection between histology or Micro-CT and all diagnostic tests. * denotes P < 0.001, ** denotes P < 0.05, NS denotes Not Significant and NA = Not Applicable.

Diagnostic accuracy for each detection method validated by both Micro-CT and histology (140 teeth): Occlusal. The mean sensitivity and specificity for each detection method for occlusal caries detection as determined by histology and Micro-CT for 140 teeth (172 investigation sites) at D_1 diagnostic threshold and D_3 diagnostic threshold are presented in Figure 4.15. The actual values obtained by each examiner and for each examination technique when histology and Micro-CT were used as validation method can be seen in Appendices 4.1 and 4.2 respectively. Note that for site specific scores/readings (ICDAS, FOTI, DIAGNOdent and CarieScan) the "site specific gold standard" was used for validation, whereas for the surface scores obtained with the radiographic techniques the "overall gold standard" was used for validation (Figure 4.15).

The performance of all detection methods for occlusal caries detection in terms of sensitivity and specificity was comparable when using either histology or Micro-CT as a validation method at D_1 diagnostic threshold and D_3 diagnostic threshold.

When using the site specific scores and readings together with the "site specific gold standard", the sensitivity values obtained with ICDAS and CarieScan PRO at the D_1 diagnostic threshold were higher than other detection methods (mean sensitivity = 78% (histology)/80% (Micro-CT) and 82% (histology)/ 81% (Micro-CT) respectively). At the D_3 diagnostic threshold, CBCT showed the best performance in relation to sensitivity values (mean sensitivity = 84% (histology and Micro-CT)) closely followed by ICDAS when the cut-off between codes 1 and 2 were used to differentiate sound and dentine caries (mean sensitivity = 70% (histology)/ 71% (Micro-CT)).

The specificity values of all detection methods were comparable at both diagnostic thresholds (mean specificities values = 77% to 95%) with two notable exceptions:

CarieScan PRO had the lowest mean specificity value at the D_1 diagnostic threshold (mean specificity = 26% (histology)/ 25% (Micro-CT)) and the DIAGNOdent which had the highest mean specificity value at the D_1 diagnostic threshold (100% with histology).

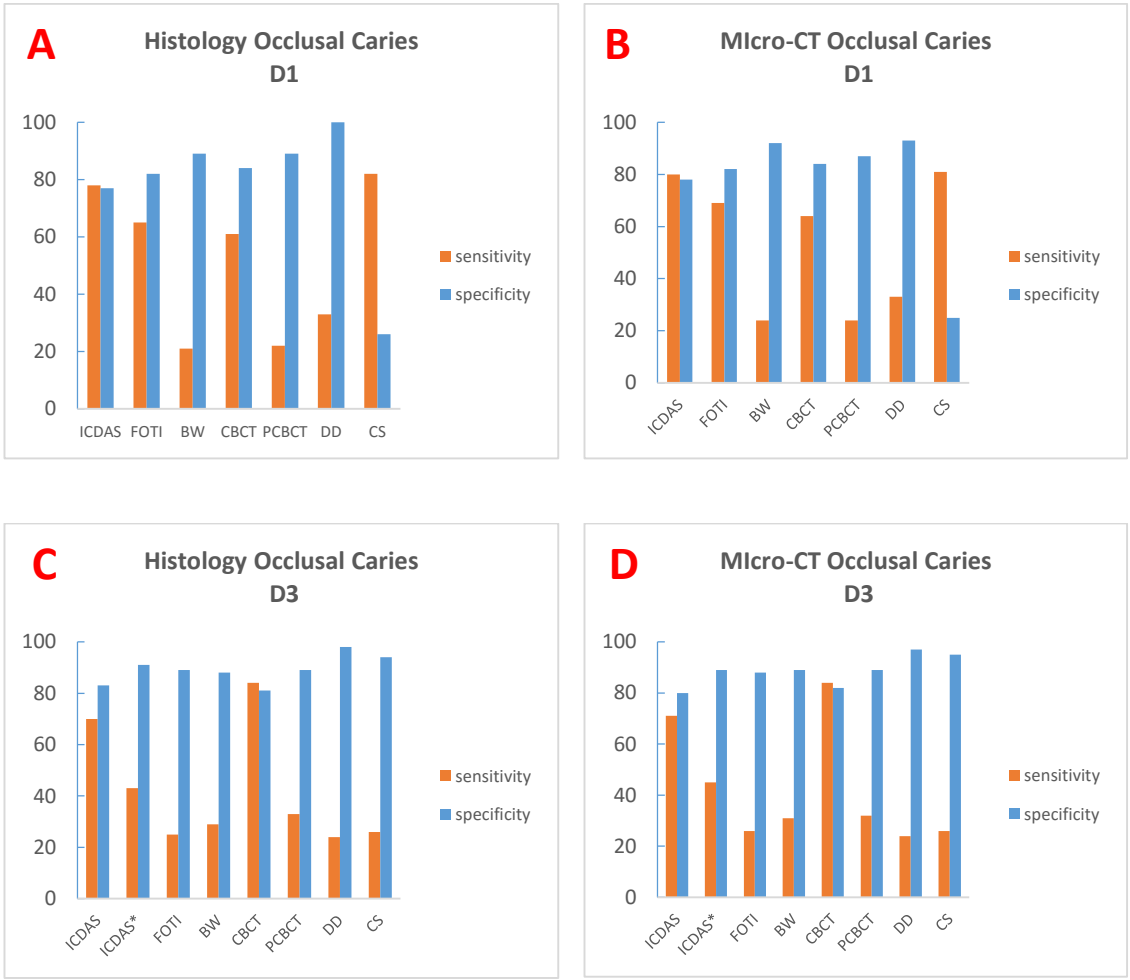


Figure 4.15 The mean sensitivity and specificity for each detection method at the D_1 (A and B) and D_3(C and D) diagnostic threshold as determined by histology (A and C) and Micro-CT (B and D) for occlusal caries including 140 teeth (172 investigation sites). ICDAS* denotes to D_3 threshold at cut-off point 2/3.

Where more than one occlusal investigation site was chosen per tooth the deepest score/reading for ICDAS, FOTI, DIAGNOdent and CarieScan was also recorded and validated with the "overall gold standard" (deepest recorded lesion if present) for direct comparison with the radiographic techniques. The mean sensitivity and specificity for these can be seen in Figure 4.16. The actual values obtained by each examiner and for

each examination technique when histology and Micro-CT were used as validation method can be seen in Appendices 4.3 and 4.4 respectively.

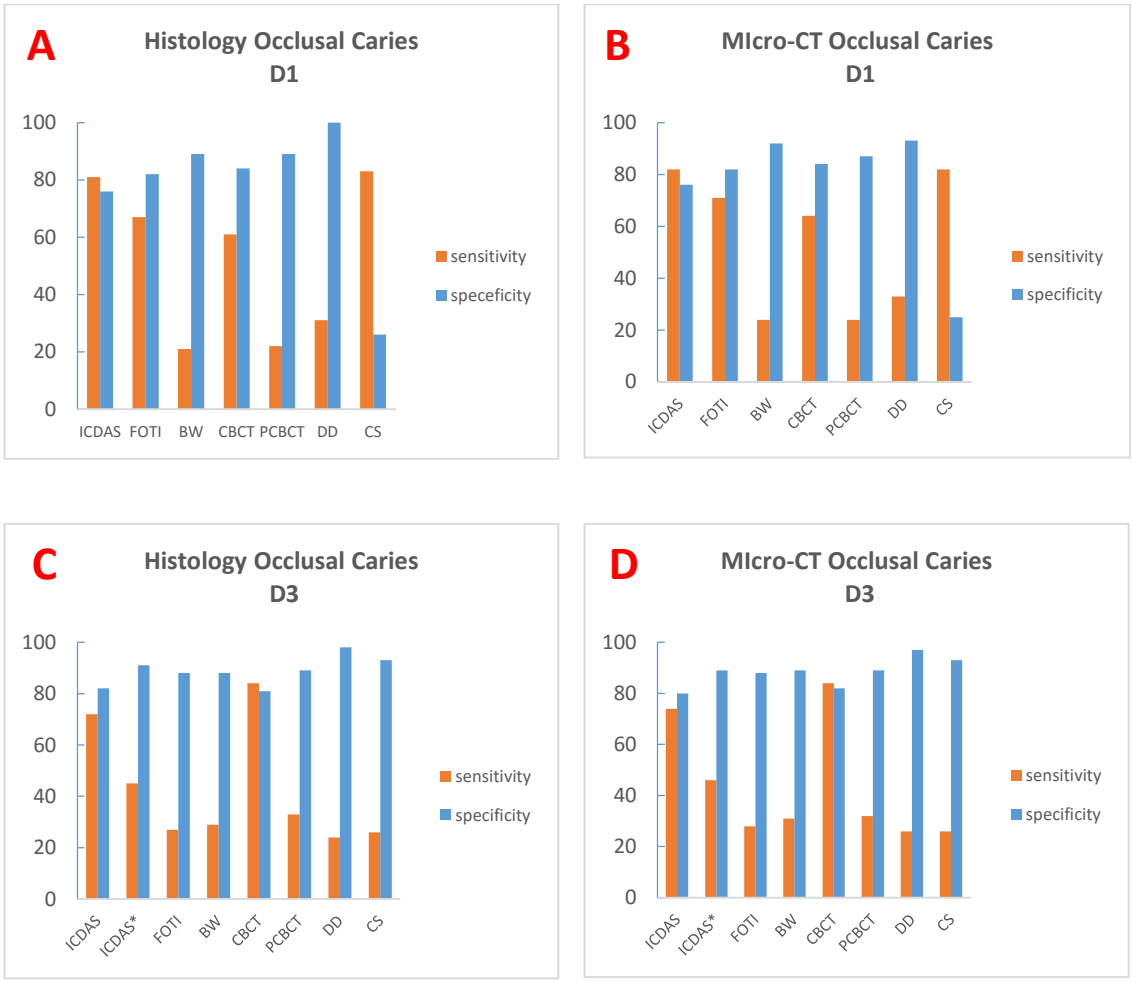


Figure 4.16 The mean sensitivity and specificity for each detection method at the D_1 (A and B) and D_3 (C and D) diagnostic threshold as determined by histology (A and C) and Micro-CT (B and D) for occlusal caries including 140 teeth based on "overall gold standard" for all examination techniques. ICDAS* denotes to D_3 threshold at cut-off point 2/3.

Figures 4.15 and 4.16 showed that when the worse score/reading for ICDAS, FOTI, DIAGNOdent Pen or CarieScan detection methods for occlusal caries detection was validated against the deepest aspect of the lesion on the occlusal surface ("overall gold standard") there was very little impact on diagnostic accuracy compared to when site specific scores/readings were used and validated against the "site specific gold standard"

(histology or Micro-CT) at both D_1 and D_3 diagnostic threshold (Figure 4.15 Site specific graph).

Diagnostic accuracy for each detection method validated by both Micro-CT and histology (140 teeth): Proximal. The mean sensitivity and specificity for each detection method for proximal caries detection based on "proximal surface gold standard" for histology and Micro-CT as validation techniques for 140 teeth (280 surfaces) at D_1 diagnostic threshold and D_3 diagnostic threshold are presented in Figure 4.17. The actual values obtained by each examiner and for each examination technique when histology and Micro-CT were used as validation method can be seen in Appendices 4.5 and 4.6 respectively.

The performance of all detection methods for proximal caries detection in terms of sensitivity and specificity was comparable using histology and Micro-CT as a validation method at the D_1 diagnostic threshold and D_3 diagnostic threshold.

The sensitivity values obtained showed that use of the DIAGNOdent Pen resulted in the lowest sensitivity (mean sensitivity = 10% (histology) / 11% (Micro-CT)) compared to the other techniques at D_1 diagnostic threshold whereas use of the digital bitewing radiograph and the DIAGNOdent Pen had the same lowest mean values at the D_3 diagnostic threshold (mean sensitivity = 11% (histology) / 12% (Micro-CT). At both D_1 and D_3 diagnostic threshold both ICDAS and CBCT resulted in the highest sensitivity values (Figure 4.17).

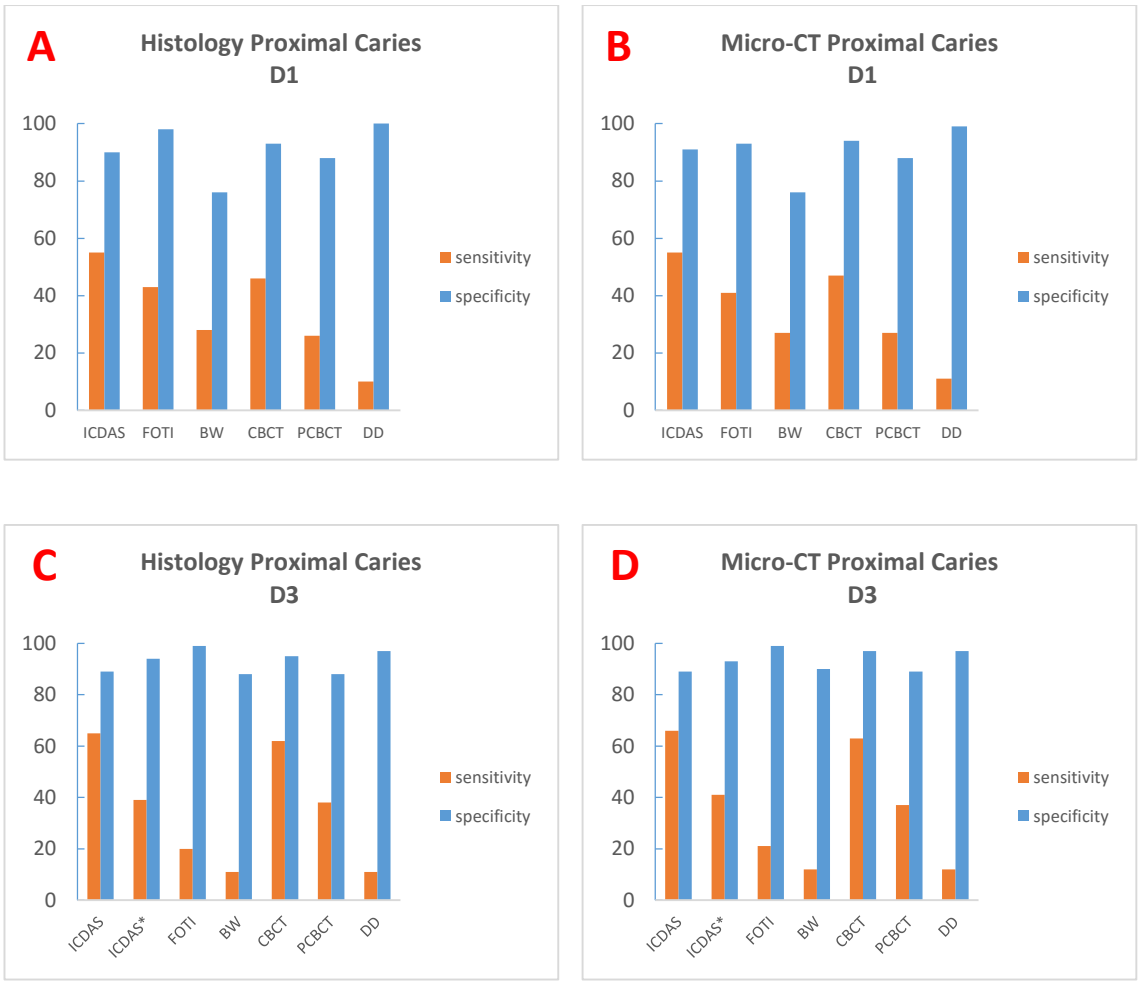


Figure 4.17 The mean sensitivity and specificity for each detection method at the D_1 (A and B) and D_3 (C and D) diagnostic threshold as determined by histology (A and C) and Micro-CT (B and D) for proximal caries including 140 teeth (280 surfaces). ICDAS* denotes to D_3 threshold at cut-off point 2/3.

The specificity values obtained by all detection methods were comparable at both diagnostic thresholds being in the range between 88% and 99% (histology and Micro-CT). However, the digital bitewing radiograph resulted in the lowest specificity value at the D_1 diagnostic threshold (mean specificity value = 76%).

The mean sensitivity values for all detection methods for occlusal caries detection were higher than those obtained for proximal caries detection at both D_1 and D_3 diagnostic threshold.

Diagnostic accuracy for each detection method validated by histology for the whole sample (200 teeth): Occlusal. The mean sensitivity and specificity for each detection method for occlusal caries detection based on the "site specific gold standard" validation as determined by histology for the 200 teeth (244 occlusal investigation sites) at D_1 diagnostic threshold and D_3 diagnostic threshold are presented in Figure 4.18. Note the site specific scores or readings for the ICDAS, FOTI, DIAGNOdent Pen and the CarieScan were validated by the "site specific gold standard" whereas the "overall gold standard" (deepest aspect of any lesion) was used for the radiographic techniques. The actual values obtained by each examiner and for each examination technique when histology was used as validation method can be seen in Appendix 4.7.

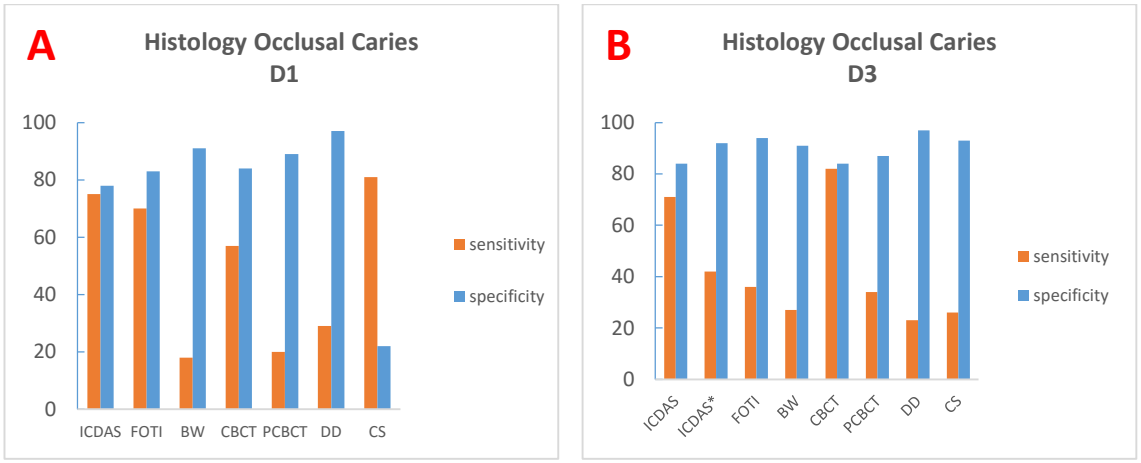


Figure 4.18 The mean sensitivity and specificity for each detection method as determined by histology at the D_1 (A) and D_3 (B) diagnostic threshold for occlusal caries including 200 teeth (244 investigation sites). ICDAS* denotes to D_3 threshold at cut-off point 2/3.

At both the D_1 and D_3 diagnostic threshold, both the sensitivity and specificity values are comparable (only varying by a maximum of +/- 5%) when the whole sample of 200 teeth were examined and validated, compared to the results obtained when the 140 teeth (that had undergone Micro-CT) were examined and validated. The only exception to this was

the sensitivity of FOTI at the D_3 diagnostic threshold which increase from 25% (for 140 teeth) to 36% for the whole sample.

Where more than one occlusal investigation site was chosen per tooth the deepest score/reading for ICDAS, FOTI, DIAGNOdent and CarieScan was also recorded and validated with the "overall gold standard" (deepest recorded lesion if present) for direct comparison with the radiographic techniques. The mean sensitivity and specificity for these can be seen in Figure 4.19. The actual values obtained by each examiner and for each examination technique when histology was used as validation method can be seen in Appendix 4.8.

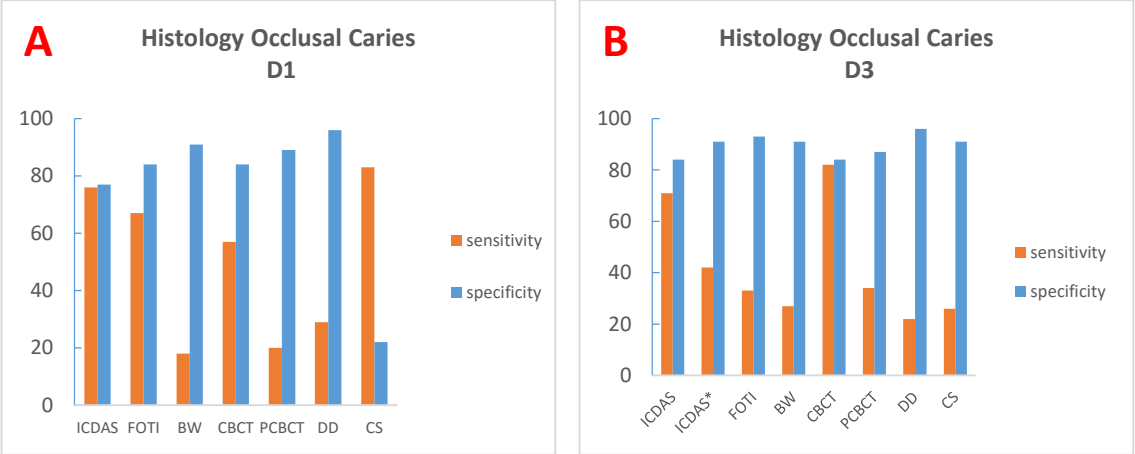


Figure 4.19 The mean sensitivity and specificity for each detection method as determined by histology at the D_1 (A) and D_3 (B) diagnostic threshold for occlusal caries including 200 teeth. ICDAS* denotes to D_3 threshold at cut-off point 2/3.

The mean sensitivity and specificity for the deepest recorded score or reading for each occlusal surface and when examined by ICDAS, FOTI, DIAGNOdent Pen and CarieScan detection methods and validated by the "overall gold standard" determined by histology for 200 teeth at the D_1 diagnostic threshold and D_3 diagnostic threshold were comparable with the values obtained when "site specific gold standard" validation was used (Figure 4.18 and Figure 4.19).

Diagnostic accuracy for each detection method validated by histology for the whole sample (200 teeth): Proximal. The mean sensitivity and specificity for each detection method for proximal caries detection based on "proximal surface gold standard" validation as determined by histology for the 200 teeth (400 surfaces) at the D_1 and D_3 diagnostic threshold are presented in Figure 4.20. The actual values obtained by each examiner and for each examination technique when histology was used as validation method can be seen in Appendix 4.9.

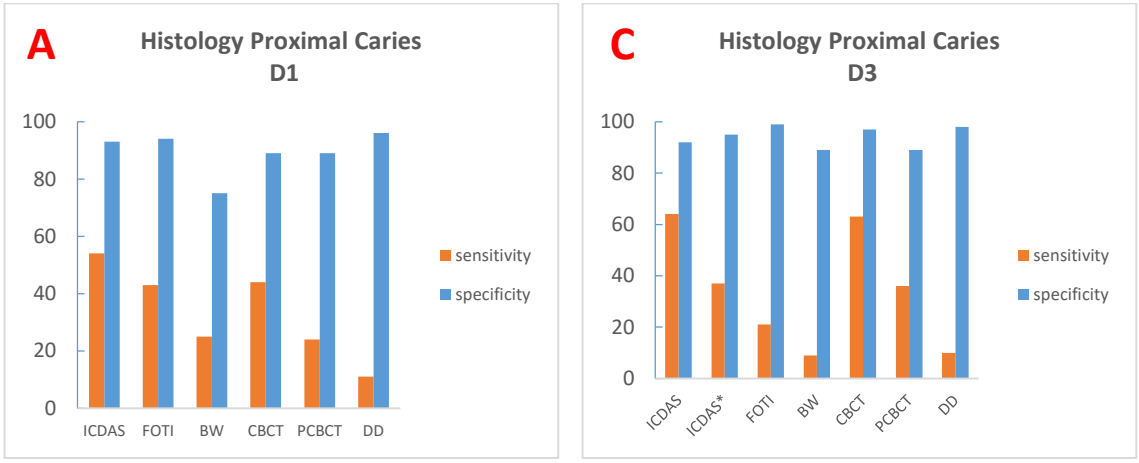


Figure 4.20 The mean sensitivity and specificity for each detection method as determined by histology at the D_1 (A) and D_3 (B) diagnostic threshold for proximal caries including 200 teeth (400 surfaces). ICDAS* denotes to D_3 threshold at cut-off point 2/3.

The sensitivity and specificity values obtained with all detection methods at both D_1 and D_3 diagnostic threshold were comparable (+/- 4% maximum) when the smaller subsample (140 that underwent Micro-CT) were compared with the data from the entire sample of 200 teeth.

Calculation of coefficients of variation for each detection method at D_1 and D_3 diagnostic threshold showed, that for occlusal caries detection, variation between examiners in sensitivity values were comparable for ICDAS, FOTI, CBCT, DIAGNOdent Pen and CarieScan PRO (5-28%) and less than that for digital bitewing radiographs (30-40%).

Variation between examiners in relation to specificity values between examiners were comparable and acceptable (0-10%).

Calculation of coefficients of variation for each detection method at D_1 and D_3 diagnostic threshold showed, that for proximal caries detection, variation between examiners in sensitivity values were comparable and higher than that for occlusal caries detection (0-42%). Variation between examiners in specificity values between examiners were comparable and acceptable (0-8%).

4.4 Discussion

Accurate caries diagnosis should be facilitated by early caries detection, lesion severity estimation and monitoring over time so that non-cavitated lesions can be treated preventively with the aim of avoiding cavitation (Pitts, 2004b). Selection of appropriate techniques for the detection of caries is therefore very important (Twetman *et al.*, 2013). The ideal diagnostic test should have high sensitivity (detects caries lesions with few false negative cases), high specificity (detects sound surfaces with few false positive cases) and should be reproducible within each operator (intra-examiner reproducibility) over time and between operators (inter-examiner reproducibility), the latter being the focus of Chapter 2 (Huysmans and Longbottom, 2004; Nyvad, 2004).

The ideal validation technique for the evaluation of caries detection methods, or so called "gold standard, should take into account the anatomical and pathological course of caries lesions and be independent of the diagnostic test evaluated. For dental caries validation, the most commonly used criterion is the depth of the lesion as estimated from the histopathological extension of the lesion (Wenzel and Hintze, 1999; Anusavice, 2005).

Two histological depth classification systems (Downer and ERK) were used in this study to determine lesion depth on occlusal and proximal surfaces from both the histological sections and Micro-CT tomographic images for comparison. In this part of the study, only the Downer classification system was used as it allows diagnostic accuracy to be investigated at both the D_1 and D_3 diagnostic threshold as it uses the EDJ as a cut-off between codes 2 and 3 (Downer, 1975).

The distribution of lesions on the occlusal surface as determined by the Downer system, shows that about a third of the investigation sites on the occlusal surface had lesions that extended into the inner half of enamel and only 28% were sound (Figure 4.2). This would mean that the preliminary inspection and selection of teeth for this study was not effective in producing a low caries prevalence sample on the occlusal surface. This having been said the prevalence of caries into dentine (D_3 diagnostic threshold) on the occlusal surface was much lower at 25%. On the proximal surface the caries prevalence was also lower (22% at the D_1 and 7% at the D_3 diagnostic threshold) and more evenly distributed through the severity codes. This is what would have been expected as teeth were selected based upon creating a low caries prevalence sample with a variety of lesions. The problem with the higher prevalence of enamel caries on the occlusal surface was mainly down to the molar teeth and the tooth type, mainly third molar teeth which would have been difficult to clean in the pits and fissures when in the mouth.

Based on the D_1 diagnostic threshold of consensus Downer scores, the caries prevalence for occlusal caries at D_1 threshold was high 72% whereas for proximal surface was 22%.

The relationships between histology, Micro-CT and caries detection methods for occlusal and proximal caries detection was moderate to strong with the exception of BW

radiographs and CarieScan Pro which had a weak relationship (with histology, BW r_s = 0.32 and CS r_s = 0.21; for Micro-CT, BW r_s = 0.35 and CS r_s = 0.23). However, for proximal caries the weakest relationship with histology and Micro-CT was for DIAGNOdent (r_s = 0.32 and 0.34 respectively) and almost negligible relationship with BW radiographs. The relationship between histology, Micro-CT and ICDAS, CBCT and FOTI for occlusal and proximal lesions were comparable. This indicates that these caries detection methods are able to assess lesion severity based upon depth of lesion on both surfaces. The percentage of agreement between histology, Micro-CT consensus scores with all caries detection methods for proximal caries were higher than that for occlusal caries. This would be expected in such a sample with low caries prevalence on proximal surfaces (313 proximal surfaces were sound).

4.4.1 Diagnostic Accuracy of Caries Detection Methods for Occlusal Caries validated with Micro-CT and Histology at D_1 and D_3 Diagnostic Threshold:

A Diagnostic threshold is a term that describes the cut-off level used to differentiate caries from no caries according to the "gold standard". Such thresholds have been used for many years (Pitts and Fyffe, 1988) and make up standardized diagnostic criteria such as those used in the Dundee Selectable Threshold Method (DSTM) (Fyffe *et al.*, 2000). Such systems allow data to be produced for caries detection methods at a variety of diagnostic thresholds, typically the D_1 diagnostic threshold where caries of enamel and dentine are classed as caries and D_3 diagnostic threshold where caries into dentine only is classed as caries and enamel lesions included with sound.

The most commonly reported outcome measures for a detection method in laboratory studies are sensitivity and specificity. These values have also been used by authors in

systematic reviews for the comparison of performance of different detection methods (Bader *et al.*, 2002; Altman and Bland, 1994; Twetman *et al.*, 2013; Gimenez *et al.*, 2015b; Gomez *et al.*, 2013a; Ismail, 2004).

The performance of all detection methods for occlusal caries detection in terms of sensitivity and specificity was highly comparable when using either histology or Micro-CT as the validation method at both D_1 and D_3 diagnostic threshold. This would mean that Micro-CT is a reliable and alternative non-destructive method of validation and comparable to histology (Kawato *et al.*, 2009; Kamburoglu *et al.*, 2011). At both D_1 and D_3 diagnostic threshold, the sensitivity and specificity values were comparable (only varying by maximum of ± 5%) when the whole sample of 200 teeth was validated by histology and the 140 teeth were validated with the Micro-CT. This would mean that the representative sample of 140 teeth that underwent Micro-CT were appropriate and representative of the whole sample.

At the D_1 diagnostic threshold, the sensitivity values for ICDAS and CarieScan PRO were higher than other detection methods for (ICDAS mean sensitivity = 0.78 for histology and 0.80 for Micro-CT; CarieScan mean sensitivity= 0.82 for histology and 0.81 for Micro-CT). On the other hand, the specificity values for all detection methods for both histology and Micro-CT were variable ranging between 1.00 for the DIAGNOdent and 0.77 for ICDAS, and CarieScan PRO which achieved the lowest value of 0.25 for histology and 0.26 for Micro-CT.

At the D_3 diagnostic threshold, CBCT and ICDAS (cut-off 1/2) were superior in relation to sensitivity values compared with other detection methods for both histology and Micro-CT (CBCT mean sensitivity = 0.84 for both histology and Micro-CT; ICDAS cut-off 2/3

mean sensitivity = 0.70 for histology and 0.71 for Micro-CT). It is noted that the sensitivity for BW, CS, FOTI and DD were considerably lower compared with histology and Micro-CT ranging from 0.24 to 0.31. However, , the specificity values for all detection methods validated by histology and Micro-CT were higher ranging between 0.98 for DD and 0.80 for ICDAS cut-off 2/3.

When the deepest score/reading for ICDAS, FOTI, DD and CS was validated with the "overall gold standard" (histology and Micro-CT) for comparison with other radiographic detection methods, there was very little impact on the diagnostic accuracy compared when site specific scores/readings were used at both D_1 and D_3 diagnostic threshold this was because in the majority of cases the deepest aspect of any lesion was deliberately chosen as one of the investigation sites.

Jablonski-Momeni *et al.*, in (2012) investigated the effect of scoring multiple lesions on occlusal surfaces, as opposed to only one representative lesion, on the reproducibility and accuracy of fluorescence-based devices. They found very little effect of measuring multiple sites on teeth with fluorescence devices compared to one site being investigated.

A recent systematic review by Gimenez *et al,* (2015b) to evaluate the overall accuracy of different visual classification systems for carious lesions detection, found that the average sensitivity and specificity values at D_1 diagnostic threshold were 0.81 and 0.73 respectively which are comparable with our results regarding the ICDAS (mean sensitivity and specificity at D_1 threshold were 0.78 and 0.77 as determined by histology). The average sensitivity and specificity values at D_3 diagnostic threshold for visual examination method were 0.59 and 0.88 respectively; in our study, ICDAS achieved higher values for sensitivity (mean sensitivity at cut-off 1/2 0.70 as determined by

histology) and comparable specificity values (mean specificity at cut-off 1/2 0.83 as determined by histology). They found that ICDAS had better performance in detecting deep lesions on the occlusal surface of permanent teeth. Another recent systematic review by Gomez *et al,* (2013a) was conducted to investigate the performance of detection methods for non-cavitated carious lesions. The visual examination for occlusal caries detection using different classification systems including ICDAS showed sensitivity values (sound versus non-cavitated lesion threshold) ranged between 0.44 and 0.83 and specificity values ranged between 0.46 and 0.90. Both sensitivity and specificity values for ICDAS at the D_1 diagnostic threshold in our study fell within these ranges. The good performance of ICDAS in this study may be due to that fact that the training and calibration of the examiners using a defined protocol was of benefit.

It is obvious in our study that ICDAS performance in terms of sensitivity at the D_3 diagnostic threshold at cut-off 1/2 was considerably higher than at cut-off 2/3 (mean sensitivity 0.43 for the latter). This would be expected as ICDAS was based on ERK classification system where ICDAS 2 (equivalent to ERK histological score 2) signifies the presence of either deep enamel or superficial dentine lesions and in our study almost half of the lesions on the occlusal surfaces were in the inner half of the enamel or outer third of dentine. Previous studies have also shown different results for sensitivity of ICDAS at D_3 threshold when both ICDAS cut-offs 1/2 or 2/3 are used and again this has been shown to be dependent on the distribution of lesions within the sample (Teo *et al.*, 2014; Qudeimat *et al.*, 2015).

It is obvious in our results regarding CS performance at D_1 diagnostic threshold that although the sensitivity was high for CS (0.82), it was at the expenses of specificity which was unacceptably low (0.25) with high numbers of false positives. The opposite was

noticed at the D_3 diagnostic threshold where the sensitivity and specificity were 0.26 and 0.94 respectively. This would raise concerns on the ability of the CS for differentiating between sound and carious lesions using the recommended cut-off readings suggested by the manufacturer. Few studies have been found in the literature regarding the performance of the CarieScan PRO. Mortensen et al. (2014) conducted an *in vitro* study to investigate the performance of CS for detecting occlusal caries on permanent teeth. Using the CS readings of 90 and above as indicating caries, the sensitivity and specificity values were 0.08 and 0.54 respectively; both much lower values than obtained in our study (0.26 and 0.94). The caries prevalence in the Mortensen et al. study was 99% (only one sound surface) and higher than in our study (72%) but despite this the sensitivity was still extremely low. Another study by Teo et al in (2014) investigated the *in vivo* and *in vitro* validity of CarieScan PRO in the detection and assessment of occlusal caries in primary teeth using the same cut-off points used in our study for the *in vitro* part of the study. The sensitivity and specificity results were found to follow the same pattern with our study (sensitivity and specificity values of CS at cut-off ≥21 were 0.97 and 0.04 respectively; at cut-off ≤91 were 0.52 and 0.87 respectively). Both previous studies highlighted an issue regarding the sensor tip of CS which is wide and tufted which was less site specific in its reading and the design of the handle made it difficult to keep steady in the fissures during use.

Regarding CBCT, only one study was found that investigated the performance of CBCT for occlusal caries detection and validated by Micro-CT (Young *et al.*, 2009). The sensitivity value obtained by CBCT for detecting lesions limited to enamel was 0.64 which is comparable with our result at the D_1 diagnostic threshold (0.61). For those lesions extending into dentine, the sensitivity was 0.93 which again is close to our results

at the D_3 diagnostic threshold (0.83). However, our specificity values at D_1 and D_3 diagnostic threshold (0.87 and 0.81) were higher than the previously reported results (0.61 and 0.63) (Young *et al.*, 2009). This could be attributed to the difference in lesion distribution within the sample and different criteria used for caries detection; our criteria being based upon extent of the lesion and that reported previously being based upon a five step confidence scale at each diagnostic threshold (Young *et al.*, 2009). In a further study by Alomari *et al*, in (2015) investigating the performance of CBCT for the detection of dentine lesions on occlusal surfaces and also using a five step confidence scale, found sensitivity and specificity values at the dentinal threshold were 0.63 and 0.64 respectively; a lower sensitivity and same specificity as in our study (0.83 and 0.64 respectively). A possible explanation for this might be the beam-hardening artefacts that appear in the dentine area as enamel is a highly dense tissue and may mislead observers in distinguishing caries lesions (Tsuchida *et al.*, 2007; Young *et al.*, 2009). On the other hand, Ertaş *et al*, (2014) also investigated the performance of CBCT for detection of occlusal caries on permanent teeth. The sensitivity values at D_1 and D_3 threshold were 0.92 and 0.81 and the specificity values were 0.67 and 0.94 respectively.

One of the problems associated with interpreting all radiographs is a tendency to make false-positive dentine caries recordings, especially on occlusal surfaces (Diniz *et al.*, 2010), due to the Mach-band effect; a perceptual phenomenon in which there is an enhancement of the contrast between a dark and a relatively lighter areas, leading the eye to believe there is a dark area underneath the EDJ (Berry Jr, 1983) and hence the recording of false positives (Espelid *et al.*, 1994).

The DIAGNOdent Pen performance was poor in terms of sensitivity at both D_1 and D_3 diagnostic threshold (0.33 and 0.24), whereas specificity values were high (1.00 and

0.98). This would mean that this method was perfect in distinguishing sound tooth structure from carious lesions, however the results in this study in relation to the DIAGNOdent should be interpreted with caution as the teeth were extracted before 2006 and stored in water which could have diluted and washed out any bacterial by products (which fluoresces and is the basis of DIAGNOdent readings) in the deeper more porous dentine lesions. In addition, it has been shown that the fluorescence values and hence the required cut-off values, decreased when the teeth were stored in thymol or formalin but remained stable when the teeth were frozen during storage and were only defrosted just before taking measurements (Francescut *et al.*, 2006).

A recent systematic review by Gimenez *et al.*, (2013c) evaluating the diagnostic performance of fluorescence-based devices for detecting caries has shown that the DIAGNOdent Pen sensitivity and specificity values for occlusal caries detection was 0.75 and 0.81 respectively at D_1 diagnostic threshold and 0.72 and 0.71 at D_3 diagnostic threshold. These findings are consistent with that found in a another recent systematic review by Twetman *et al.*, in (2013) looking at the performance of adjunctive methods for caries detection on permanent and primary teeth. The results of DIAGNOdent showed that the mean sensitivity and specificity (SD) of DIAGNOdent for occlusal caries detection was 0.66 (SD = 0.29) and 0.77 (SD = 0.19) respectively at D_1 diagnostic threshold and 0.72 (0.20) and 0.82 (0.16) at D_3 diagnostic threshold. They suggested that the DIAGNOdent could therefore be a useful adjunctive method to visual examination for occlusal caries detection in permanent teeth.

At the D_1 diagnostic threshold the performance of FOTI showed better results for sensitivity (0.65) than at the D3 diagnostic threshold (0.25), however the specificity values were comparable at both D_1 and D_3 thresholds (0.82 and 0.89 respectively). The

performance of FOTI was comparable or slightly lower than ICDAS for enamel and dentine caries detection on occlusal surfaces. It seems that the examiners were not able to detect dentinal lesions and this could be attributed to the difficulty in distinguishing enamel and dentine lesion shadows even after the modification of the classification system used for FOTI examinations in this study. The poorer sensitivity at the D_3 threshold may also be due to the fact that the examiners were not familiar with the technique in their daily practice and the fact that there is so much surrounding tooth tissue that the light has to pass through and which could mask subtle shadows in pits and fissures.

Bader *et al,* in (2002) conducted a systematic review on the performance of different methods for caries detection and found only three studies using FOTI for occlusal caries detection. The sensitivity for detection of enamel and dentine lesions ranged between 0.14 and 0.74 whereas the specificity ranged between 0.85 and 0.95, in agreement with our findings and those in a systematic review by Twetman in (2013). The authors of these systematic reviews found insufficient evidence for the use of FOTI for occlusal caries detection.

The digital BW performance was very poor in terms of sensitivity at both D_1 and D_3 diagnostic threshold (0.21 and 0.29 respectively), whereas specificity values were considerably higher (0.92 and 0.88 respectively). The BW method performed well in terms of specificity with less false positive cases compared with ICDAS (0.78) at D_1 diagnostic threshold.

The examiners were not able to detect either enamel or dentine lesions on the occlusal surface even though the quality of the images was superior with no soft tissue equivalent material. This is due to the size of the lesions included and the amount of sound buccal

and lingual enamel to the fissures, that attenuates the x-ray beam. False positives were also not an issue in this study for BW examination, as a result of the Mach band phenomenon and this is most likely due to a high negative caries response rate in general, due to the difficulty and doubt the examiners had for caries detection on the occlusal surface. Previous studies have also reported such low sensitivites using digital radiographic systems for enamel lesion detection (0.19 (Ashley *et al.*, 1998) and 0.16 (Wenzel *et al.*, 1990)) and dentine lesion detection (0.31 (Haiter-Neto *et al.*, 2008) and 0.34 (Alomari *et al.*, 2015)).

4.4.2 Diagnostic Accuracy of Caries Detection Methods for Proximal Caries validated with Micro-CT and Histology at D_1 and D_3 Diagnostic Threshold:

As with occlusal caries the performance of all detection methods for proximal caries detection in terms of sensitivity and specificity values was highly comparable when using either histology or Micro-CT as validation method at both the D_1 and D_3 diagnostic threshold. This would mean that Micro-CT is also a reliable and alternative non-destructive method compared with histology for validation of proximal caries detection methods (Soviero *et al.*, 2012; Tsuchida *et al.*, 2007)

At both D_1 and D_3 diagnostic threshold, the sensitivity and specificity values were comparable (only varying by maximum of ± 4%) when the whole sample of 200 teeth were validated by histology and the 140 teeth were validated with histology or Micro-CT. This would mean that the representative sample of 140 teeth was appropriately selected with various appearances for comparing the two validation methods.

At the D_1 diagnostic threshold, the sensitivity values for ICDAS, CBCT and FOTI were considerably higher than other detection methods (ICDAS mean sensitivity 0.55 (when

validated by both histology and Micro-CT); CBCT 0.46 (histology) and 0.47 (Micro-CT); FOTI 0.43 (histology) and 0.41 (Micro-CT)), although lower than that achieved for occlusal caries detection. Specificity values for all detection methods for both histology and Micro-CT were relatively high, ranging between 1.00 for DIAGNOdent Pen and 0.76 for digital BW radiographs.

At the D_3 diagnostic threshold, ICDAS (cut-off 1/2) and CBCT were superior in relation to sensitivity values compared with other detection methods for both histology and Micro-CT validation (ICDAS 0.65 (histology) and 0.66 (Micro-CT); CBCT 0.62 (histology) and 0.63 (Micro-CT). Sensitivity values for BW, PCBCT, FOTI and DD were considerably lower ranging from 0.38 to 0.11 and specificity values for all detection methods were high ranging from 0.99 for FOTI and 0.89 for ICDAS (cut-off 1/2).

When the deepest score/reading for ICDAS, FOTI, DD and CS was validated with the "overall gold standard" (histology and Micro-CT) for comparison with other radiographic detection methods, there was very little impact on the diagnostic accuracy compared when site specific scores/readings were used at both D_1 and D_3 diagnostic threshold.

A recent systematic review by Gimenez *et al,* (2015b) to evaluate the overall accuracy of different visual classification systems for proximal caries detection found that the average sensitivity and specificity values for *in vitro* studies at the D_1 diagnostic threshold were 0.72 and 0.79 respectively which are slightly higher than our sensitivity results with ICDAS (mean sensitivity = 0.55) and lower than our mean specificity (0.90) as determined by histology. They also found that the average sensitivity and specificity values at the D_3 diagnostic threshold for visual examination method were 0.44 and 0.93 respectively. In our study, ICDAS achieved a higher value for sensitivity (mean

sensitivity (cut-off 1/2) = 0.65) and comparable specificity (mean specificity (cut-off 1/2) = 0.89). They suggested that the variations among *in vitro* studies could be due to the difficulty of simulating proper contacts between teeth which would influence whether the proximal surfaces were seen or not (Braga *et al.*, 2009a). In our study, the lower sensitivity at the D_1 diagnostic threshold may arise from the low caries prevalence on the proximal surface and inclusion of a high proportion of shallower lesions, together with the fact that a "soft tissue" gingival mask was used to simulate the clinical situation with more difficult visual access. Despite the slightly lower sensitivity at the D_1 diagnostic threshold, the higher specificities (as found in our study) are preferred in a population with low caries prevalence to avoid overtreatment especially at the dentinal threshold so avoiding unnecessary operative intervention (Downer, 1989).

A further systematic review by Gomez *et al,* (2013a) was conducted to investigate the performance of detection methods for non-cavitated carious lesions. The visual examination for proximal caries detection using different classification systems including ICDAS showed sensitivity values (sound versus non-cavitated lesions) ranged between 0.17–0.22 and specificity values ranged between 0.88 and 0.95. The sensitivity values were considerably lower than our sensitivity value (0.55) and those reported by Gimenez *et al*, (2015b), however specificity values were comparable with what achieved by ICDAS at D_1 diagnostic threshold. The reason for this is unclear as there would have been a degree of overlap in the included studies; it may very well be down to the inclusion criteria (sound versus non-cavitated lesions as opposed to caries severity scales as in our study).

It is obvious in our study that ICDAS performance in terms of sensitivity at the D_3 diagnostic threshold at cut-off 1/2 was considerably higher than at cut-off 2/3 (mean

sensitivity 0.39). However, the specificity values were comparable. The explanation for this is identical to that related to occlusal caries discussed earlier.

Few studies have investigated the performance of CBCT for proximal caries detection on permanent teeth. Young *et al.,* in (2009) and Haiter-Neto *et al.,* in (2008) investigated the diagnostic accuracy of CBCT for proximal caries detection and validated with Micro-CT and histology. They found that the sensitivity and specificity for lesions limited to enamel ranged from 0.18 to 0.24 and from 0.85 to 0.95 respectively. For lesions extending into dentine the sensitivity and specificity ranged from 0.44 to 0.61 and from 0.89 to 0.94 respectively. This is in close agreement with our results at D_1 and D_3 diagnostic threshold. It seems that the performance of CBCT for detection of dentine lesions is better than shallow lesions in enamel (Cheng *et al.*, 2012; Zhang *et al.*, 2011). As such if CBCT radiographs are taken for other reasons it is important that the status of the proximal tooth surface is examined and reported upon.

The mean sensitivity and specificity values for FOTI at the D_1 and D_3 diagnostic threshold 0.43 and 0.98 respectively at the D_1 threshold and 0.20 and 0.99 at D_3 diagnostic threshold. This would mean that the performance of FOTI was better in the detection of enamel and dentine lesions rather than dentine lesions only. On the contrary, higher sensitivity values have been reported previously for FOTI and proximal caries at the D_3 diagnostic threshold ranging from 0.67 to 0.85 (Mialhe *et al.*, 2009; Mitropoulos, 1985; Peers *et al.*, 1993). These differences in the performance of FOTI could be explained by variations in the experience of the examiners, the ordinal scale used for identification of lesions, the low caries prevalence in our study for dentine lesions and the validation methods used as the gold standard.

The digital BW performance was very poor in terms of sensitivity at both D_1 and D_3 diagnostic threshold (0.28 and 0.11), whereas specificity values at the D_3 diagnostic threshold were acceptable (0.88) and at the D_1 threshold relatively poor (0.76) with about a quarter of sound surfaces being incorrectly diagnosed as carious. The latter could be explained by commonly occurring cervical burnout on proximal surfaces (Berry Jr, 1983). The poor sensitivity is more difficult to explain as the quality of the images was good as there was no soft tissue equivalent, so improving image contrast. Whilst digital radiographs have a significantly lower resolution than conventional radiographs which consist of millions of grayscale values, this is unlikely to explain the low sensitivities. Indeed research and previous systematic reviews have also confirmed lower than expected sensitivities ranging between 0.17 and 0.36 and moderate specificites ranging from 0.76 and 0.91 for the detection of proximal lesions (Haiter-Neto *et al.,* 2008; Huysmans *et al.,* 1997; Mitropoulos *et al.*, 2010).

The DIAGNOdent Pen's performance was very poor in terms of sensitivity at both the D_1 and D_3 diagnostic threshold (0.11 and 0.10 respectively), whereas specificity values were excellent (1.00 and 0.97 respectively) keeping false positives to a minimum. The reasons attributed to such results have been explained in the previous section (4.5.1) and they should be regarded with caution. Of note, the examiners found this technique difficult and time consuming; hence only three examiners used the technique in this study for proximal caries. Using the new wedge shaped tip it was not always possible to detect lesions under inaccessible proximal contacts where access was difficult. It has also been suggested that caries detection of proximal tooth surfaces with DIAGNOdent Pen might be influenced by the condition of the adjacent tooth surface (Lussi *et al.,* 2006b).

In a recent systematic review by Gimenez *et al.*, (2013c), conducted to evaluate the performance of fluorescence-based devices for detecting caries showed that the DIAGNOdent Pen sensitivity and specificity for proximal caries detection was 0.77 and 0.84 respectively at the D_1 diagnostic threshold and 0.75 and 0.82 at D_3 diagnostic threshold.

The variation between examiners for each detection method in terms of sensitivity values for proximal caries was higher than that for occlusal caries. It is obvious that the low caries prevalence in the sample for proximal lesions affected the variation between examiners making lesion detection much more difficult.

4.5 Conclusions

Within the limitations of the present study, the conclusions that could be drawn were:

1. The relationship between histology, Micro-CT and caries detection methods for occlusal and proximal caries detection was moderate to strong with the exception of digital BW radiographs and CarieScan Pro which had a weak relationship for occlusal caries. For proximal caries the weakest relationship with histology and Micro-CT was for DIAGNOdent Pen and almost negligible with digital BW radiographs.
2. The performance of all detection methods for occlusal and proximal caries detection in terms of sensitivity and specificity was highly comparable when using either histology or Micro-CT as the validation method at both D_1 and D_3 diagnostic threshold. Based on this finding it can be concluded that Micro-CT is a reliable and alternative, non-destructive method compared with histology for validation of caries detection methods.

3. For occlusal caries detection, ICDAS and CarieScan PRO were superior at the D_1 diagnostic threshold, whereas CBCT and ICDAS were superior at D_3 diagnostic threshold in terms of sensitivity compared to other detection methods. With the exception of the CarieScan PRO which had an unacceptably low specificity value, the specificity values for all other detection methods were clinically acceptable.
4. For proximal caries detection, ICDAS and CBCT were superior at both diagnostic thresholds in relation to sensitivity. However, with the exception of the bitewing radiograph, specificity values for all detection methods were clinically acceptable and above 0.88.
5. The performance of DIAGNOdent Pen and digital BW radiographs were poor in terms of sensitivity at both diagnostic thresholds for occlusal and proximal caries detection.
6. The performance of FOTI was poor in terms of sensitivity at D_3 diagnostic threshold for occlusal and proximal caries detection.

CHAPTER FIVE

Quantitative Analysis of Occlusal and Proximal Carious Lesions as Determined by Micro-CT: An *In vitro* Study

5.1 Introduction

Dental caries is a disease process that occurs in the plaque biofilm on the surface of the tooth, the first sign of which is the white spot enamel lesion: demineralisation affecting the hardest biological tissue in humans (Fonseca *et al.*, 2008). Microscopically the early carious lesion has a natural three-dimensional complex structure (Shimoda *et al.*, 2008) attributed to its dynamic process of demineralization and remineralization (Zavgorodniy *et al.*, 2008; Featherstone, 2004) which if left unchecked can progress into dentine and lead to cavity formation. However, when considering caries research, most researchers have considered caries lesion extent in terms of lesion depth in a two dimensional manner. When evaluating lesion extent in the laboratory most methods require destruction of the tooth in order to obtain a thin slice or serial sections of the tooth to allow an internal view of the lesion and internal tooth structures usually under a microscope (Kawato *et al.*, 2009). Thus, there is a disparity and potential misunderstanding of the carious lesion when researchers try extrapolating findings from a two dimensional image of complex three dimensional carious lesion and related tooth structures (Wong *et al.*, 2006).

Micro-Computerised Tomography (Micro-CT) is a non-destructive 3D imaging system which can be used to qualitatively determine lesion extension (Mitropoulos *et al.*, 2010; Soviero *et al.*, 2012) and quantitatively assess the mineral densities of dental hard tissues through attenuation of x-ray photons (Dowker *et al.*, 2004; Gao *et al.*, 1993). Although Micro-CT is a valuable method for analysing lesion depth by investigating individual tomographic cuts according to specific subjective criteria (Dos Santos *et al.*, 2015), it could be more useful to explore the area of a lesion i.e. depth and width of the lesion to investigate lesion extension in relation to the surrounding structures.

Micro-CT is an emerging method which allows three dimensional recording of inner structures with high spatial resolution and the ability to create virtual sections in all dimensions and to segment dental hard tissues based on the mineral loss (Swain and Xue, 2009). It therefore has the potential to provide in-vitro volumetric quantification of caries lesions based on the mineral density of hard tooth structures. However Micro-CT has only been used previously to validate methods of caries removal based upon the mineral density of carious and sound tooth structures and the expected volume of caries excavated (Neves *et al.*, 2011; Zhang *et al.*, 2013). Previously volumetric analysis of carious lesions has only been possible following caries removal and assessment of the dimensions of a silicone impression of the cavity (Lennon *et al.*, 2007).

Whilst there has been a wide range of Micro-CT studies, none has explored the characteristics and heterogeneity of natural carious lesions in human teeth as three dimensional objects and attempted to determine the volume of enamel and dentine lesions while preserving the tooth structure. The only attempt known to investigate the volume of carious lesions was carried out by Shimoda *et al*, (2008) on provoked lesions in 20 mice which were divided into a control group fed with a sucrose free diet and an experimental group fed with 30% sucrose containing diet. Both groups were exposed to cariogenic bacteria. The mean volume of the control group teeth was subtracted from the volume of each tooth in the experimental group to calculate the caries volume.

Micro-CT is also a useful method for accurately mapping the mineral concentration in sound enamel and dentine (Huang *et al.*, 2007; Djomehri *et al.*, 2015), and determining the mineral concentration of natural or artificial enamel white spot lesions (Cochrane *et al.*, 2012; Lo *et al.*, 2010). It also has the potential to explore the relationship between the area of the enamel lesion and its mineral loss, as Micro-CT has the ability to provide an

accurate insight into changes associated with mineral density within caries affected enamel (Elliott *et al.*, 2008; Schwass *et al.*, 2009).

This *in vitro* study therefore aimed to:

1. Devise a quantitative method for area and volume analysis of occlusal and proximal lesions from Micro-CT images.
2. Develop a novel method to determine the percentage mineral loss in the enamel part of the lesion at its deepest aspect on occlusal and proximal surfaces.
3. Determine the area of the lesion, volume of the lesion if present and the percentage mineral density loss at the deepest part of the lesion on both the occlusal and proximal surfaces as determined by the Downer classification system on the 2D tomographic image.
4. Determine the relationship between the area of the lesion, volume of the lesion and the mineral density content within the enamel lesion at the sites with the deepest lesion and the, whether in enamel or extending into dentine on both the occlusal and proximal surface as determined by the Downer classification system on the 2D tomographic image.
5. Determine the relationship between the area of the lesion, volume of the lesion and the mineral density loss within the enamel lesion at the sites with the deepest lesion on occlusal and proximal surface with the consensus scores of ICDAS, FOTI, Radiographic examination methods, DIAGNOdent Pen and CarieScan PRO results.

5.2 Materials and Methods

5.2.1 Study Sample and Selection of Examiner:

Only 140 teeth underwent Micro-CT examination. The consensus Micro-CT data derived by the two examiners used for the Micro-CT and histological examinations in Chapter 3 were used in this study. Using the Downer classifications, on the occlusal surface the deepest aspect of a lesion beneath an investigation site was used for the site specific caries detection examinations and the deepest aspect of any lesion on the occlusal surface, if different, were investigated (Chapter 4). On the proximal surface the deepest aspect of the lesion only was investigated.

One examiner who was a qualified experienced dentist with at least 15 years post graduate experience devised a novel method to calculate the total volume of enamel lesions and dentine lesions for those extending beyond the EDJ.

5.2.2 Micro-CT Scanning Technique:

The Micro-CT data derived for the 140 teeth investigated out of the entire 200 teeth used in Chapter 3 and 4 were used in this Chapter. The teeth were scanned using a small-angle-cone-beam desktop μCT 40 Scanco system (Scanco MedicalAG, Bassersdorf, Switzerland), at a linear isotropic resolution of 20 μm. The integration time was set at 2.5 seconds, 500 projections taken over 180̊ (at 0.36° angle increments) and 2 frames per projection. The duration of the full scan time was dependant on the size of the examined teeth and ranged from 2 to 3.5 hours.

A sintered hydroxyapatite disk prepared at Dental Physics Sciences Department, Queen Mary, University of London (Figure 5.1) was placed on the top of each embedded tooth sample before scanning for standardization of Micro-CT images, so avoiding any

systematic error due to potential x-ray spectrum shift between scans, and to allow quantitative assessment of mineral density of sound and carious tooth structures which will be discussed later in this Chapter. Reconstruction of the images was carried out by cone-beam algorithms in 1024 x 1024 pixels matrices. All 1024 x 1024 pixels images were acquired in Digital Imaging and Communication in Medicine (DICOM) 16 bit images. Evaluation of the images was performed using **Image J** (public domain Java image processing program by NIH Image; see http://rsbweb.nih.gov/ij/appendix.html) on a 21 inch TFT computer screen.

On the occlusal surface the Micro-CT tomographic slice representing the deepest aspect of a lesion at the selected investigation site (the same slice used as the "site specific gold standard" in Chapter 4) together with the tomographic slice of the deepest lesion on the occlusal surface, if different (the same slice used as the "overall gold standard" in Chapter 4), were used to calculate the area and the percentage mineral loss within enamel. On the proximal surface the tomographic slice with the deepest aspect of the lesion if present was chosen for identical analysis. The volumetric analysis was also carried out for the occlusal lesions related to the "site specific gold standard" and the "overall gold standard" together with all proximal lesions if present.

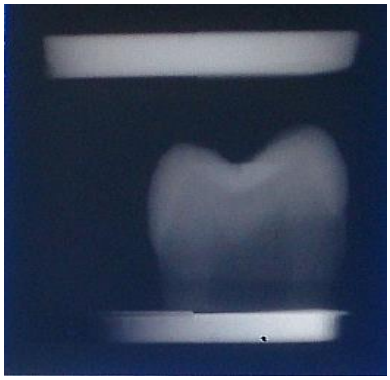


Figure 5.1 Micro-CT "scout view" of molar tooth attached to the composite triangle and hydroxyapatite disk on the top.

5.2.3 Area of the Lesion and Volume Rendering based on the Segmentation of Sound and Carious Tooth Structure:

This part of the study explores measurement of the area of a carious lesion from two dimensional tomographic images corresponding to the deepest aspect of the lesion on occlusal and proximal surfaces. It also develops a method to fully exploit the 3D information provided by the Micro-CT to quantitatively assess the volume of carious lesions which more realistically reflects the degree of tooth tissue destruction. This method was presented at an international cariology conference (ORCA, 2014) and the abstract can be found in the Appendix 5.1.

Segmentation of sound and carious tooth structure: The imported 16 bit DICOM image (grayscale values -32768 to 32767, where negative values were not physically meaningful and deleted because of detector noise) data stacks of each tooth with occlusal and/or proximal lesions were opened with **Image J.** A duplicate stack of the image slices from each tooth was created in order to split the slices representing the whole tooth from the slices representing the hydroxyapatite disk. The grayscale values corresponding to the background noise (surrounding embedding resin etc.) were eliminated from the Micro-CT images. To further enhance lesion identification the contrast was set at approximately 20%. The slices were filtered further with a median filter (radius 1 pixel) to help noise reduction on the images. Reslicing of the tooth in 0.1 mm thick virtual slices in a mesio-distal plane was then carried out. The slice thickness was chosen to reduce the number of the slices per tooth to enable meaningful analysis of the carious lesion in a manageable way.

Each pixel in an image slice is assigned a grayscale value corresponding to the linear attenuation coefficient and hence the radiopacity of the dental hard tissue and the

surroundings. Since, the mineral loss and hence radiopacity and grayscale value of sound enamel and dentine are different, identification of carious lesions within each tooth tissue was carried out separately so that appropriate grayscale thresholds could be applied for each tissue. To set the thresholds for each tooth tissue a slice representing sound enamel and dentine in the buccal and /or lingual region of the tooth was chosen. Thresholding was accomplished for each individual tooth by visualization and using the segmentation function in Image J which defines the range of gray values corresponding to the sound enamel, dentine, carious tissues and background.

For analyses within enamel, the images were converted into an 8 bit (inverted Look Up Table (LUT)) binary (black and white) image with pixel size 0.02x0.02 mm^2. Pixels with a gray scale value above the minimum grayscale value of sound enamel were allocated a white colour, grayscale values below this were assigned a black colour, this would include dentine and the background embedding acrylic, and also any lesion in enamel. Therefore the enamel cap at this stage will appear as a white structure. If an enamel lesion were present toward the surface of the enamel it would appear black and the surface of the tooth/lesion would not be visible against the black back ground (Figure 5.2 A). Therefore to allow the area and volume of a lesion in enamel to be calculated during the segmentation process, grayscale values below the lowest value for dentine were also assigned a white colour (Figure 5.2 B). This therefore also makes the background white, but due to the differences in density between the enamel and background embedding acrylic the surface of the enamel can be seen as a dark line due to the Partial Volume Effect (PVE) (Huang *et al.*, 2007).

For analyses within dentine, the images were converted into an 8 bit (inverted LUT) binary images with pixel size 0.02x0.02 mm^2. Pixels with a gray scale value above the

minimum grayscale value of sound dentine including sound enamel were allocated a white colour, grayscale values below this were assigned a black colour, this would include pulp and the background embedding acrylic, and also any lesion in dentine (Figure 5.3 A). If an enamel lesion were present and extended to the EDJ the enamel would also appear black (Figure 5.3 B). Therefore to allow the area and volume of a lesion in dentine to be calculated during the segmentation process, any lesion in enamel above the EDJ was ignored. Whilst the volume of discrete shallow dentine lesions was measured and attributed to a discrete clinical investigation site, where deeper dentine lesions are concerned the dentine lesion on occasions merged with those originating from other sites with enamel caries above, where this occurred the volume of the whole dentine lesion was measured.

All the binary images were further investigated by one examiner to determine the slices representing the start and the end of the lesion corresponding to the chosen occlusal site and proximal surface. Any lesions fewer than five pixels in the two dimensional tomographic images were excluded from the volume of carious lesion and considered as noise (Taylor *et al.*, 2010). Furthermore, the binary images for enamel and dentine lesions were visually compared with the 16 bit gray scale images to further confirm the beginning and the end of the lesion. For each tooth all binary images were stored as an image sequence or stack in separate files for each occlusal and/or proximal lesion in TIFF format.

Image processing for area and volume measurement using Adobe Photoshop CS6:

The Photoshop CS6 version 13.0.1 was used for analysis of the binary images and further determination of the area and volume of enamel and dentine lesions. Each image sequence or stack was opened by the Photoshop software programme and each slice was changed

into Red, Green and Blue (RGB) colour mode with a red and green colour replacing the black and white colour respectively to easily identify the pixels representing the carious lesion (Figure 5.4 A and B). All parts of the lesion from each slice in the image stack were selected and cropped by using the Photoshop **"Quick"** and **"Magic Wand"** selection tool which is highly sensitive in selecting all the pixels corresponding to the carious lesion (Figure 5.5 A, B and C).

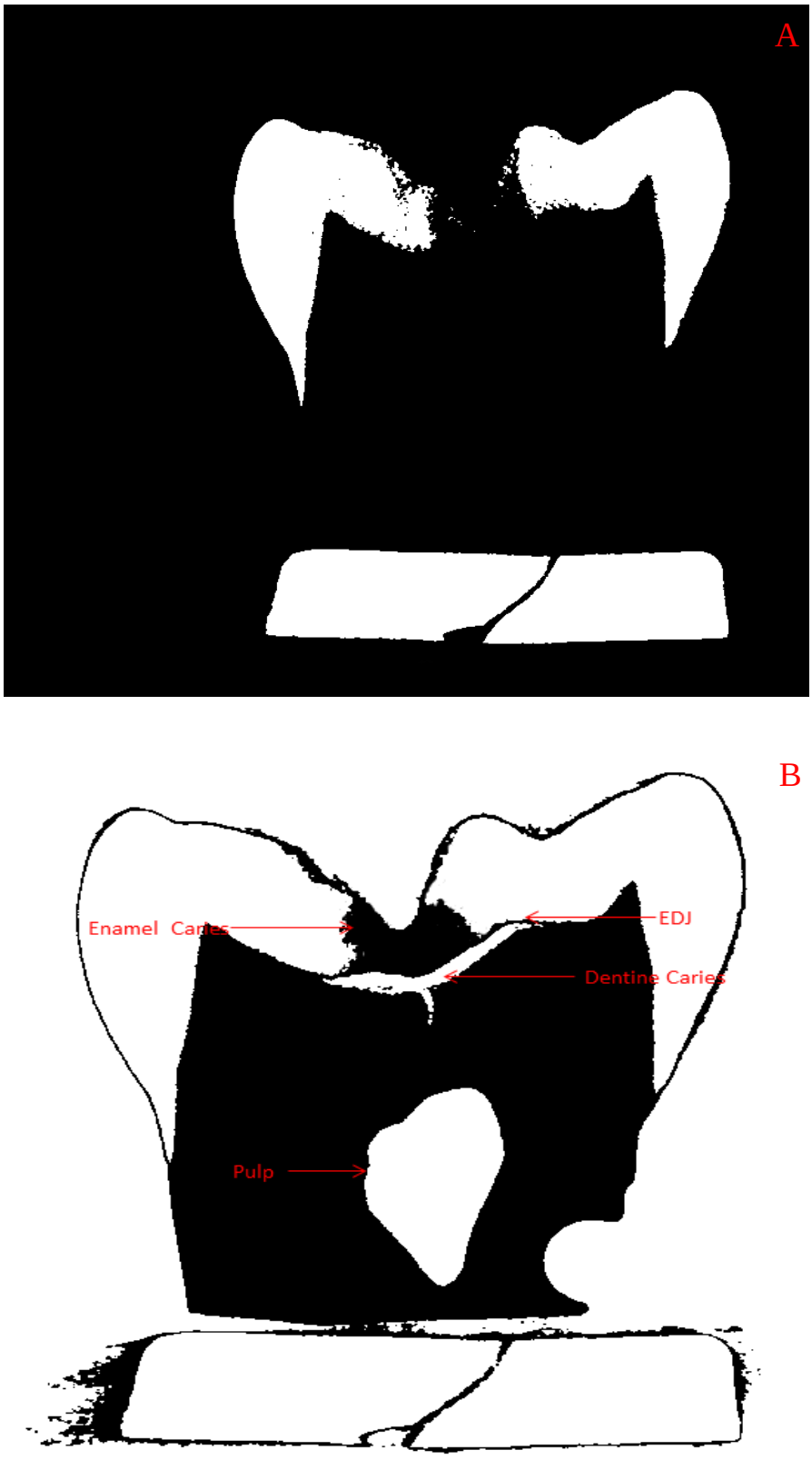
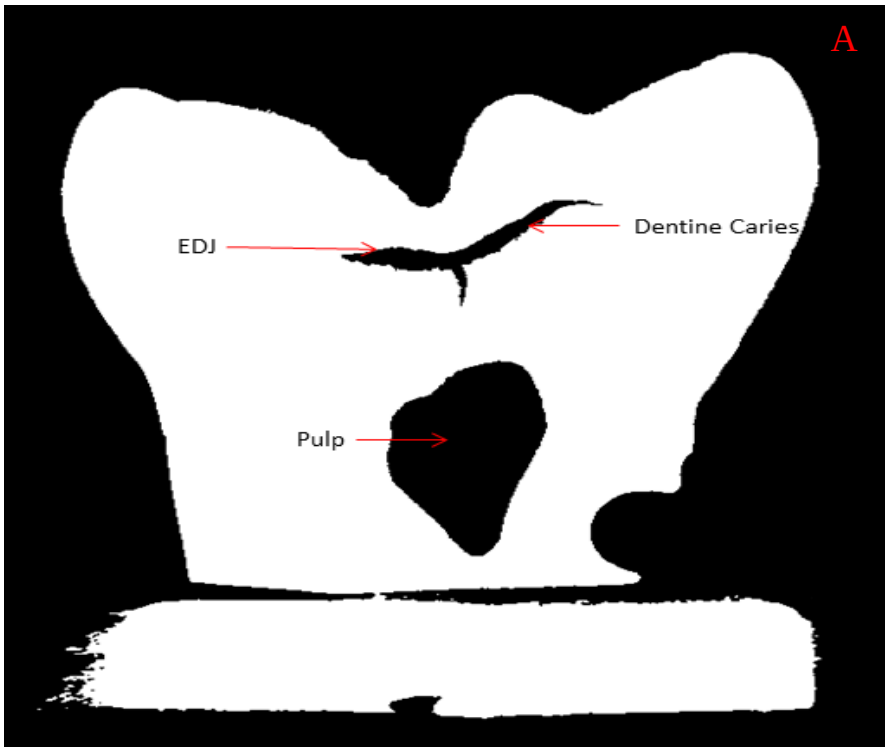


Figure 5.2 Tomographic slice representing enamel caries lesion with black background (A) and the same slice with a white background (B) after segmentation process.



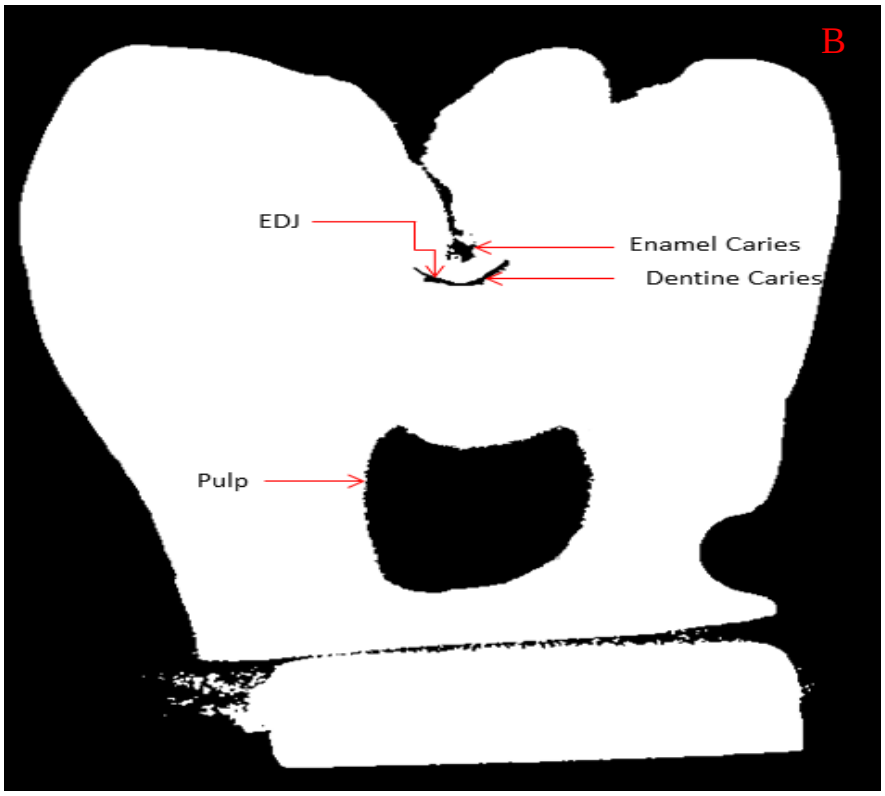


Figure 5.3 Tomographic slice representing dentine caries lesion with no enamel lesion above and with black background (A) and another slice of a different tooth with an enamel lesion above the dentine lesion showing the EDJ after segmentation process (B).

The script for each lesion was saved in TXT format consisting of the count of white and black pixels in each slice with the total volume of the lesion in cubic millimetre. Where a lesion extended into dentine, the volume of the dentine lesion was determined in the same way. The volume of the entire lesion was then calculated by adding the volume of dentine lesion to that of the enamel lesion.

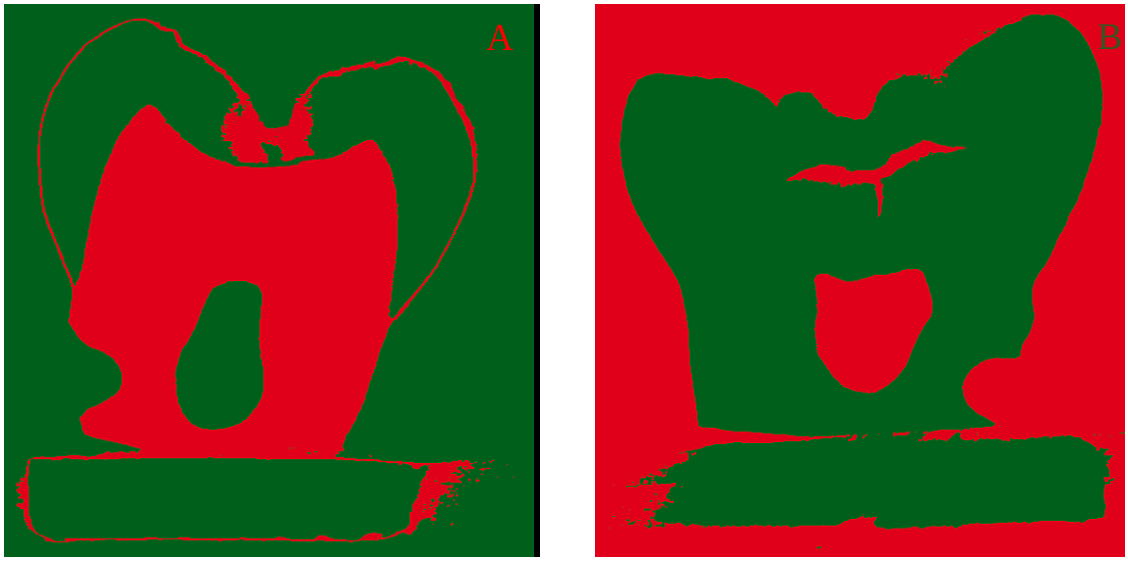


Figure 5.4 Tomographic slice (RGB) mode with enamel lesion (A), and dentine lesion (B) on occlusal surface.

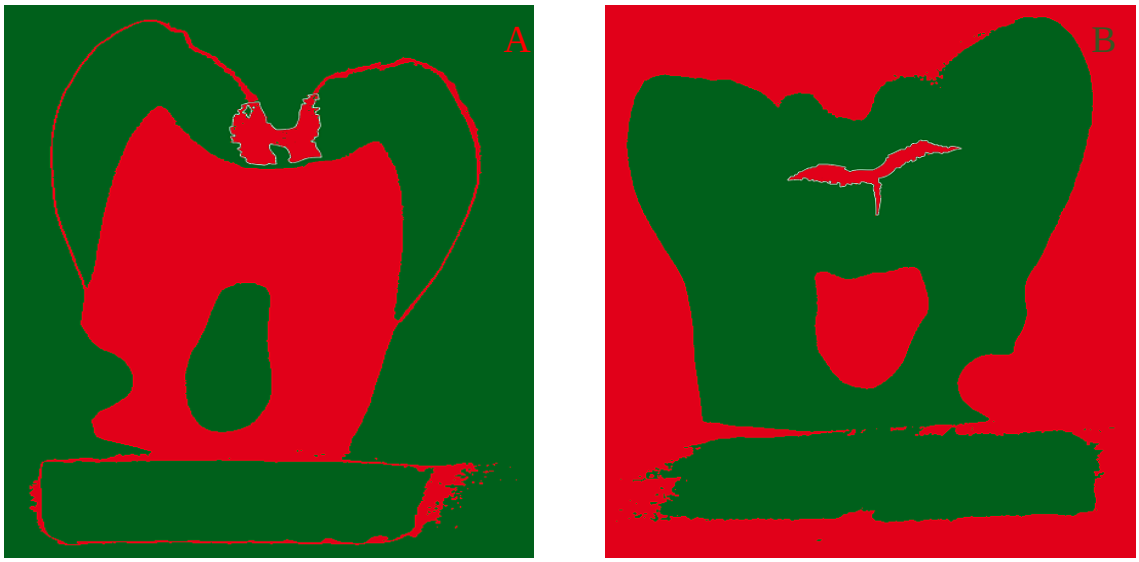


Figure 5.5 Tomographic slice (RGB) mode with selected area of enamel lesion (A), and selected area of dentine lesion (B) on occlusal surface.

Each cropped part of the lesion from each slice was then saved in a separate Photoshop **"8 bit Channel"** composed of two colours; a white colour representing the cropped carious lesion and black colour for background (Figure 5.6 A and B). To eliminate the partial volume effect along the boundaries of the cropped binary image in the channels, the grayscale threshold was set to 128 (50%), which means any pixel at the lesion boundary with a grayscale value above 128 was set to 255 (white shade) as carious and any pixel with grayscale value below 128 was set to 0 (black shade) as background.

Measurement of the area of the lesion: On the occlusal surface, the channel obtained for the Micro-CT tomographic slice representing the deepest aspect of a lesion at the selected investigation site together with the tomographic slice of the deepest lesion on the occlusal surface, if different, and any dentinal lesion if present were used to calculate the area of the lesion using Photoshop CS6. On the proximal surface, the channel obtained for the Micro-CT tomographic slice with the deepest aspect of the lesion if present (enamel and dentine) was chosen for identical analysis.

The measurement scale on Photoshop was adjusted so that each pixel was equivalent to 0.02x0.02 mm^2 as the original isotropic resolution of 20 microns.

Volumetric analysis of the lesion: A modified Photoshop **"Script"** (series of commands) was developed by a computer support specialist based at the college of Art and Design, Dundee University for volume analysis of a lesion (Appendix 5.2). It was designed to manipulate each cropped binary image series within the "Channels". Essentially the area of each cropped image was calculated from the 0.02mm by 0.02mm pixels, the tomographic slice was set at 0.1mm thickness, hence the volume of each cropped image is known. By adding the volume of each cropped slice representing the

lesion together the volume of the entire lesion was calculated. The volume of the lesion is therefore represented by the following simplified equation:

(Total volume in millimeter^3 = ∑ Total number of white Pixel * 0.02 *0.02 *0.1)

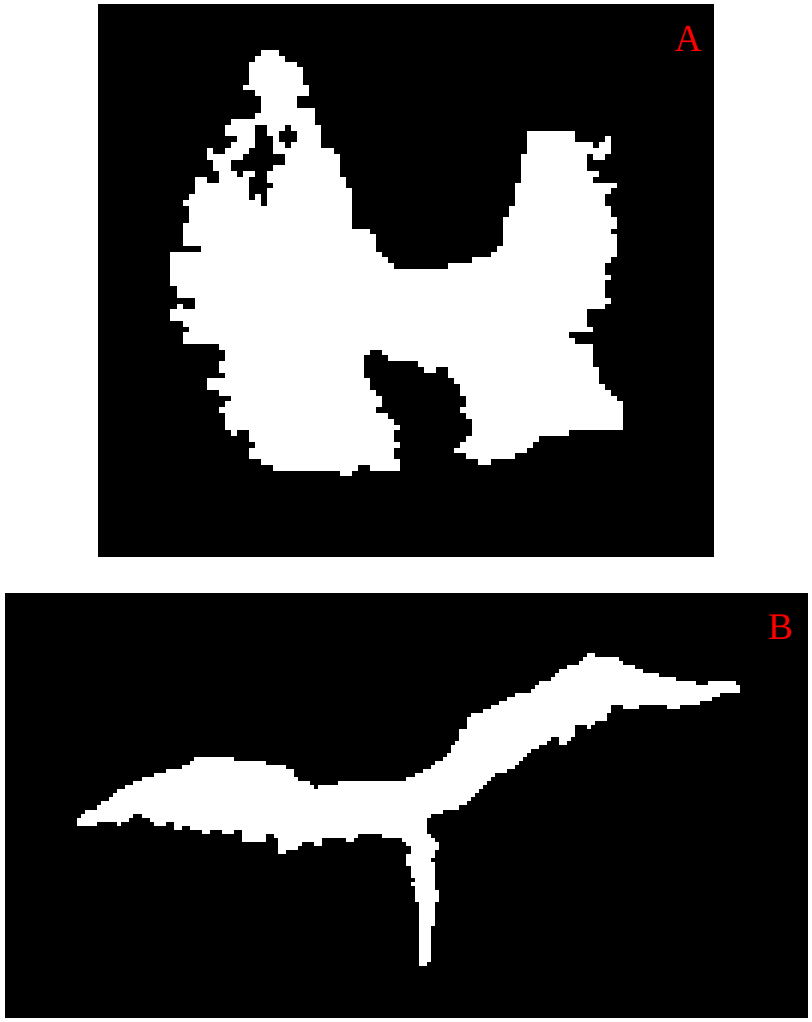


Figure 5.6 Channel of the slice representing enamel lesion (A) and channel of the slice representing dentine lesion (B).

5.2.4 Measurement of the Mineral Density of Enamel Lesion at the Deepest Part of Carious Lesion:

In this section of the study, whether a carious lesion extends into dentine or not, the mineral density of the enamel lesion only was analysed and was expressed as percentage of the mineral loss of sound enamel tissue.

Mineral Density Calibration: The "μCT 40" Scanco system used in this study, whilst allowing mineral density calculation, it is not as accurate as another scanner, called the MuCAT 2 scanner. This scanner is based and designed at Queen Mary, University of London and has the ability to calculate the absolute hydroxyapatite mineral density of enamel. The MuCAT 2 scanner has the ability to provide high contrast resolution tomographic scans and accuracy in mineral concentration quantification. However, to produce such an image from the MuCAT 2 takes a scan time in excess of 9 hours, as such a MuCAT 2 scan of a pure hydroxyapatite disc was used to calibrate the μCT 40 Scanco system which can produce acceptable images at a much lower scan time (approximately 3 hours).

The MuCAT 2 machine is characterised by using high dynamic range CCD cameras with time –delay integration readout and a modelling approach to beam hardening correction with automatic calibration using built-in multi-element carousel made of aluminium, titanium and copper of different thicknesses following each scan (Davis and Elliott, 1997; Davis and Elliott, 2003; Davis *et al.*, 2013).

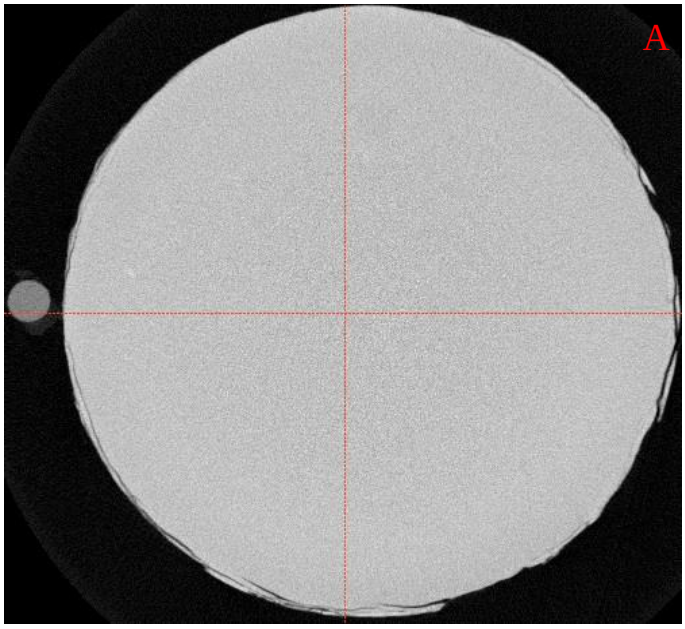
Using a sintered hydroxyapatite disk of 12 mm in diameter and approximately 2.5 mm thick having similar x-ray attenuation properties to sound enamel were scanned using the MuCAT 2 machine. The disk was scanned at 90 kVp and 0.26μA current with a pixel size of 15 microns; 1581 projections were acquired over a period of 9hr and 45 min. All images were reconstructed using the Feldkamp cone-beam (Feldkamp *et al.*, 1984).

A pure aluminium wire of 1 mm thickness was placed longitudinally next to the hydroxyapatite disk of unknown density and exposed simultaneously as a reference material that was visible in every slice to cut off the softest x-rays, so as to achieve a detector response close to 40 KeV.

The scan, produced 189 image slices (8 bit grayscale images with grayscale values 0 to 255) captured over 360º and was viewed using specifically developed software called Tomview version 1.1 (Graham Davis, Queen May, University of London, personal communication). The grayscale value for each pixel was converted to Linear Attenuation Coefficient (LAC) by dividing the grayscale value by 75 (Figure 5.7 A). This step of dividing the grayscale value by 75 to create the LAC was an integral part of the Tomview 1.1 software. A histogram of grayscale levels for the whole hydroxyapatite disc was created from the reconstructed image using **Image J** software (Figure 5.7 B). The density of the disk was calculated by dividing the mean LAC by the mass attenuation coefficient of hydroxyapatite (0.9877 cm^2g^{-1} according to 'XCOM database' which is a web database which can be used to calculate photon cross sections for scattering, photoelectric absorption and pair production, as well as total attenuation coefficients, for any element, compound or mixture ($Z \leq 100$), at energies from 1 keV to 100 GeV.) (Berger, 2010). It was found that the density of the hydroxyapatite disk was 2.58g_{Hap}/cm^3.

The same hydroxyapatite disc was placed on top of each embedded tooth during the μCT 40 Scanco scan which, now knowing the mineral density of the hydroxyapatite disc, allows more accurate mineral density calibration from the resultant images. According to the information from the manufacturer of μCT 40 Scanco system, the native grayscale values of the 16 bit grayscale images was -32768 to 32767, where negative values were not physically meaningful and deleted because of detector noise. The grayscale value for each pixel was converted to LAC by dividing on a scale 4096. The LAC values for the disk were calculated after each tooth scan and were converted later into mineral concentration assuming the mineral of the enamel was pure hydroxyapatite (3.15 g/cm3) (Zou *et al.*, 2009).

To facilitate grayscale values of enamel to be converted into absolute mineral densities, a plot was constructed of the of the mean grayscale value of the hydroxyapatite disk, measured by the μCT 40 Scanco machine , compared with the density (g/cm^3) of the disk as measured by the MuCAT 2 machine. It is reasonable that any relationship between two methods would pass through the origin of the graph. For simplicity, a linear relationship was chosen for the calculation equation used to convert the LAC values of enamel into absolute mineral density (Figure 5.8). Based on the calibration point which is the mean grayscale value of the hydroxyapatite disk as measured by the MuCAT system, the calibration equation was calculated using a fitted linear equation of y = ax+b (where y represents the mineral density in g/cm^3, x the grayscale value and a and b are the constants of the linear equation Figure 5.8). Based on the determined parameters a and b, the mineral density was calculated using the grayscale value (Farah *et al.*, 2010; Zou *et al.*, 2009). The grayscale values for the enamel were converted into LACs (cm^{-1}) values and then transformed into mineral density of HAP (g/cm^3) using the calibration equation obtained from the disk scanned on the top of each tooth.



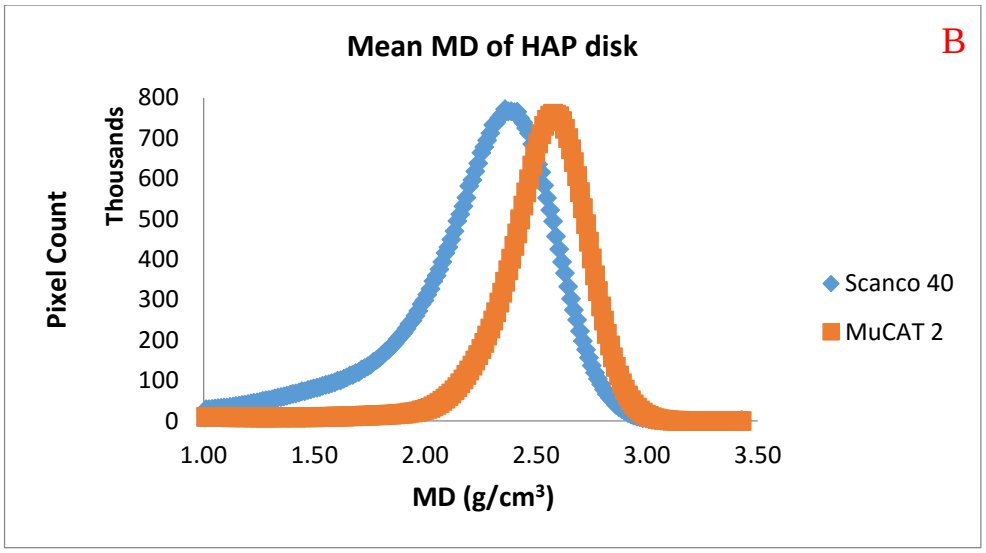


Figure 5.7 Tomographic image of Hydroxyapatite disk using Tomview software with one mm thickness Aluminium wire (A) and histogram of the Mineral Density (MD) of the disk using MuCAT 2 machine and Scanco 40 when scanned on the top of the tooth (B).

As noted in Figure 5.7 B the hydroxyapatite disk was homogenous at the micrometre level using the MuCAT 2 system (as indicated by the low standard deviation of LAC and even distribution around the mean with an un-skewed, bell-shaped curve).

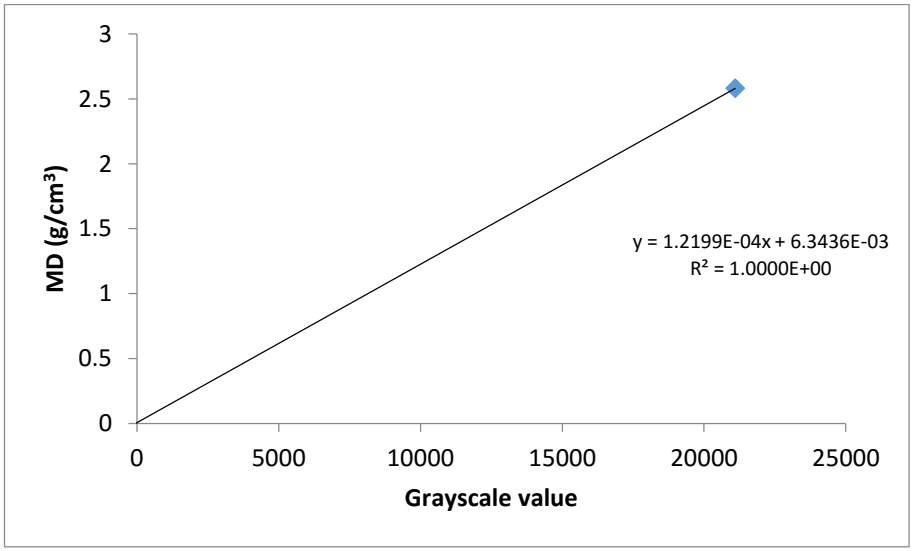


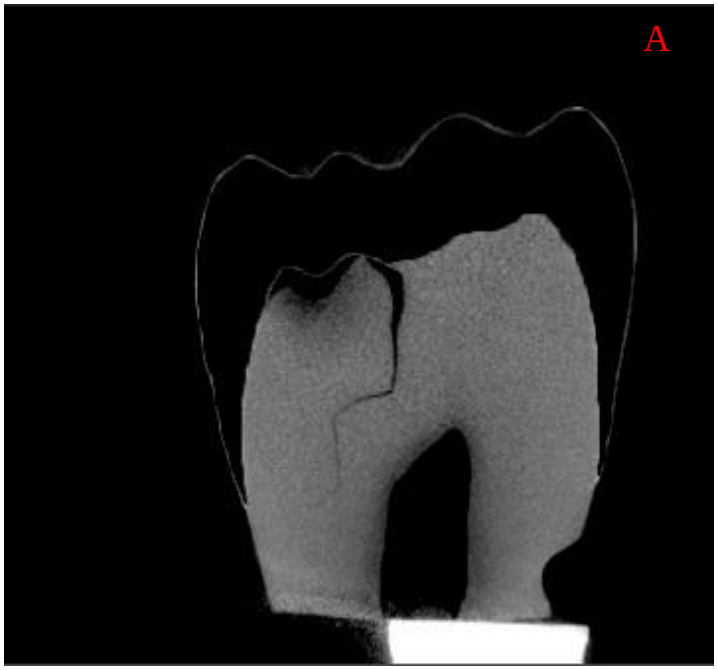
Figure 5.8 Plot of the mean grayscale value of the Hydroxyapatite disk as measured with the µCT 40 Scanco machine compared with the density of the disk as measured by the MuCAT 2 system.

Mineral Density Measurement of Sound Enamel: A slice of the original 16 bit grayscale images from the middle of the sound buccal and/or lingual enamel of each tooth

was chosen. A duplicate was created as an 8 bit grayscale image and was imported into **Photoshop CS6 software** where the section including the sound enamel was cropped using quick selection tool and saved in TIFF format (Figure 5.9 A).

These images were opened with **Image J** and further determination of the edges of the whole enamel was performed using "Wand" tool excluding the edges to avoid errors due to the Micro-CT partial volume effect (Dowker *et al.*, 2003; Farah *et al.*, 2010) (Figure 5.9 B).

A histogram of grayscale values was created for the selected region of sound enamel of each tooth and further plotted against their LACs to calculate the mineral density (g/cm^3) values of sound enamel using the linear equation determined through the calibration exercise. The mean mineral density for sound buccal and/or lingual enamel was considered as the mineral density of sound enamel for each tooth and was used to compare with the mineral density of the carious enamel lesion/s (if present) on the occlusal and proximal surface.



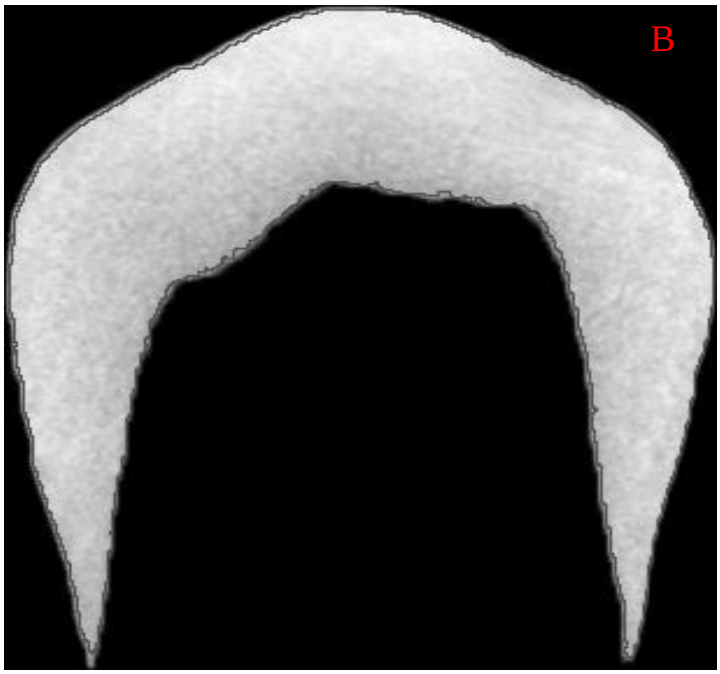
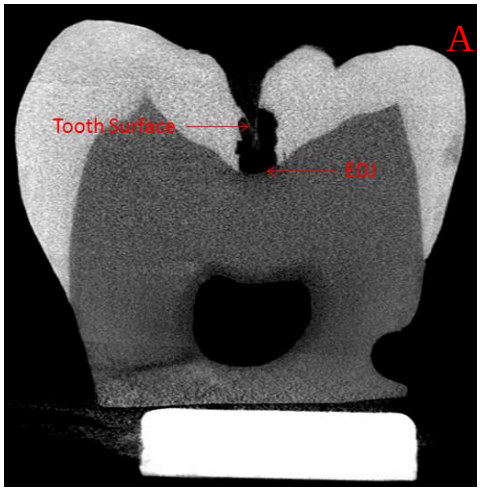
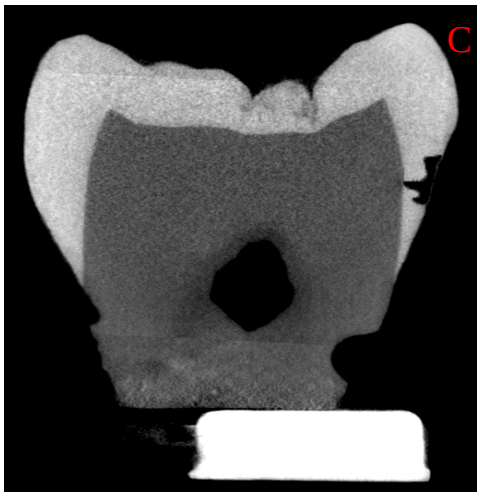


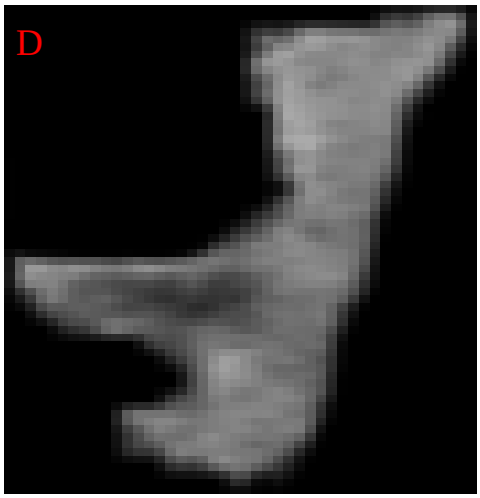
Figure 5.9 An image of a tooth after cropping and removing the sound enamel cap associated with a dentine lesion using Photoshop CS6 software (A) and an image of cropped sound enamel cap (of a different tooth) opened in Image J (B).

Mineral Density Measurement of the enamel lesion: The channel obtained using Photoshop CS6 representing the enamel lesion of the deepest aspect of a lesion at the selected investigation site or the deepest lesion on the occlusal surface, if different, and the deepest aspect of the lesion if present on the proximal surface was used to calculate the Mineral Density of the affected enamel lesion. For this the channel was duplicated and superimposed over the corresponding 16 bit grayscale tomographic image representing the Micro-CT tomographic slice to act as a **mask**. This **mask** was created to represent the dimensions of the lesion based on the threshold setting used for area and volumetric analysis rather than subjectively as appeared in the image. The mask was also used for precise selection of the area of the lesion to be measured, in such a way to exclude the pixels related to the outside edges of the lesion, surface of the tooth and EDJ to avoid the partial volume effect (Figure 5.10 A, B, C, D, E and F).

The image of the lesion on the Micro-CT tomographic slice was cropped and imported into **Image J** as an 8 bit grayscale image where a histogram was created of all grayscale values. The data was plotted in Microsoft office Excel software against their LACs cm^{-1} and converted into the equivalent mineral density using the calibration equation created from the hydroxyapatite disk. The lesion was defined as the pixels where the mineral loss was between 20% and 95% that of sound enamel (Ten Cate *et al.*, 1996). The mean mineral density of the lesion was calculated and these values were subtracted from the mean mineral density of sound enamel for that tooth. These values were expressed as percentages and will further be refered as percentage of mineral loss in occlusal and proximal lesions.







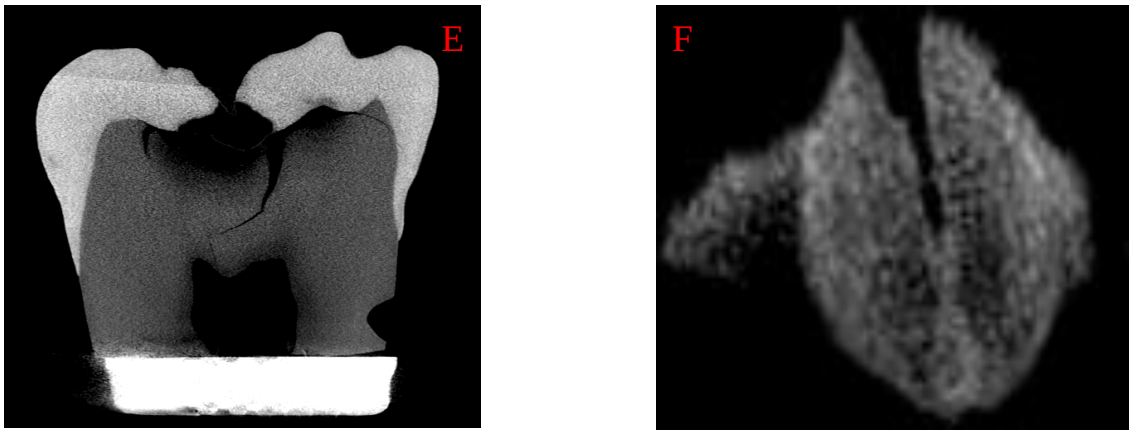


Figure 5.10 Tomographic image of the deepest aspect at the selected occlusal investigation site with cropped enamel lesion showing the tooth surface and EDJ (A) and the cropped enamel lesion (B); tomographic image of the deepest aspect of lesion on proximal surface with cropped enamel lesion (C) and the cropped enamel lesion (D); tomographic image of the deepest aspect of the lesion at the selected occlusal investigation site with cropped enamel lesion from the associated dentinal lesion (E) and the cropped enamel part of the lesion (F).

5.2.5 Statistical Analysis:

Consensus detection methods (Pooled Data): To compare the outcomes of the detection methods with lesion area, volume and mineral loss, consensus diagnostic scores or readings were determined. For detection methods with categorical scores (ICDAS, FOTI and all radiographic techniques) the consensus score for each selected investigation site and each surface was determined as the most frequent score (mode) among examiners and the frequency was expressed as a percentage. For detection methods with continuous data (DIAGNOdent Pen and CarieScan PRO) the mean of all eleven examiners readings was taken as consensus. Only the scores or readings at the deepest investigation site (as determined by the Downer score on Micro-CT) on the occlusal surface were used in the subsequent analyses. For the radiographic assessments on the occlusal surface, the consensus scores from the 11 examiners were compared with the Micro-CT data obtained from the deepest aspect of any lesion in that surface. The continuous data obtained with DIAGNOdent Pen and CarieScan PRO were also converted into categorical data according to Table 2.6 and 2.7 respectively in Chapter 2.

Sample characteristics: Shapiro-Wilk's normality test ($p< 0.001$) (Shapiro and Wilk, 1965) and a visual inspection of the data relating to volume of the lesions, the area of the lesion and the percent of mineral loss at the deepest aspect of the lesion showed that the data were not normally distributed. As such non-parametric methods were used for statistical analyses. All the statistical tests were done using SPSS software V 21(PASW statistics, SPSS Inc., Chicago, USA). The level of significance was set at $P <0.05$.

Distribution of the volume, area and mineral content loss of the occlusal and proximal lesions: Boxplots were used to summarize the distribution of the area of lesion, the volume of lesion, and percent of mineral loss (within the enamel lesion) in the lesion according to each Downer classification score for the deepest occlusal investigation site and proximal surface.

Relationship and comparison of the volume and area of the occlusal and proximal lesions and its mineral loss with consensus Downer classification system and the relevant consensus scores of caries detection methods: The relationship between the area of lesions, volume of lesions and percentage of mineral loss (within the enamel lesion) at the most affected occlusal investigation site and proximal surface according to each Downer classification score, was determined by Spearman correlation coefficient (r_s) and was illustrated using scatter plots.

The Spearman correlation coefficient (r_s) was used to examine the relationship between the consensus reading from the raw data for DIAGNOdent Pen and CarieScan PRO at the deepest occlusal investigation sites and proximal surfaces with the area, volume of the deepest lesion and the percentage of the mineral loss.

For the Downer scores and all of the detection methods (consensus scores), including the converted categorical data collected from DIAGNOdent Pen and CarieScan Pro, the Kruskal-Wallis (non-parametric) test was used to determine whether there were any statistically significant differences in the area of the lesion, volume and mineral loss in enamel according to each classification system. Where significance was found a follow-up analysis (Dunn-Bonferroni post hoc test) was undertaken to determine where the differences of the aforementioned parameters occurred between scores for each detection method. For the follow up analysis to evaluate consensus Downer scores, the zero scores were removed from the analysis. Since the zero group had no variance, it added no new information to the analysis and served only to undermine the power of the test. Additionally the presence of the zero group made the post hoc pairwise comparisons less sensitive since the post hoc tests become less powerful the greater the number of analyses conducted.

5.3 Results

Consensus detection methods (Pooled Data): At each investigation site the eleven examiners reached agreement on the score for each detection method between 60 to 88% of the time. The only two exceptions to that were for DIAGNOdent pen readings on proximal surfaces where agreement on categorical scores reached 95% and for CarieScan PRO on occlusal surfaces where agreement reached only 50%.

The distribution of consensus Downer scores, as determined by Micro-CT for 140 teeth, showed that, for the deepest occlusal investigation site per tooth, 52 (37%) sites scored zero, 23 (16%) sites scored one, 19 (14%) sites scored two, 36 (26%) sites scored three and 10 (7%) sites scored four. Similarly, the distribution of consensus Downer scores for

the 280 proximal surfaces showed that 195 (70%) surfaces scored zero, 33 (12%) surfaces scored one, 23 (8%) surfaces scored two, 23 (8%) surfaces scored three and 6 (2%) surfaces scored four.

Distribution of the volume, area and mineral content loss of the occlusal and proximal lesions: For each Downer classification the frequency distribution (median, minimum and maximum) of the area of lesions, the volume of lesions and percentage of mineral loss (in enamel) in the lesion for the deepest occlusal investigation site and proximal surface is illustrated by boxplots in Figure 5.11 and 5.12 respectively.

It is clear from Figure 5.11 and 5.12 that as the lesion become deeper according to Downer consensus score that the area of the lesion becomes larger as does the volume and percentage mineral loss in enamel (see statistical analysis later).

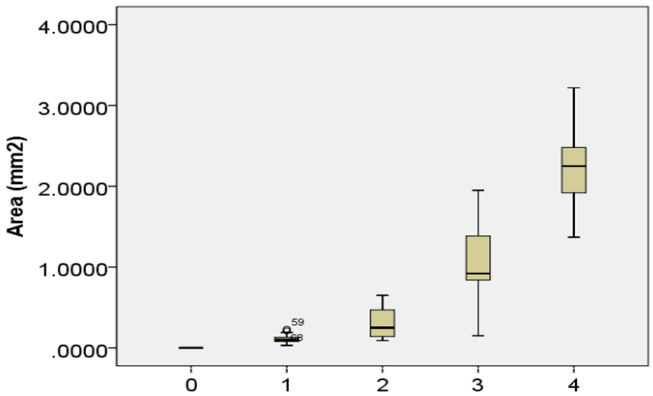
Relationship and comparison of the volume and area of the occlusal and proximal lesions and its mineral loss with consensus Downer classification system and the relevant consensus scores of caries detection methods:

The relationships between the area of lesions, volume of lesions and percentage of mineral loss (within the enamel) at the most severely affected occlusal investigation site and proximal surface is illustrated in Figure 5.13 and 5.14 respectively.

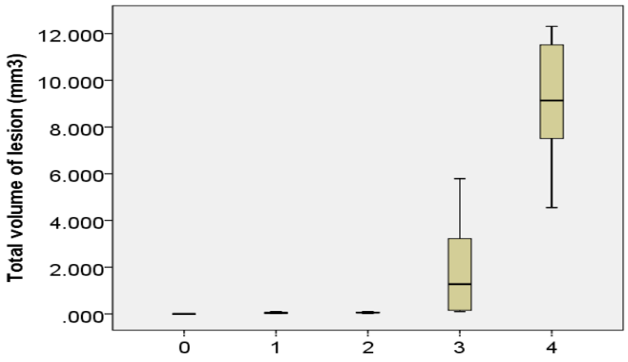
A very strong direct relationship was found between the variables area of the lesion, volume of the lesion and the percentage of mineral loss within the enamel at the most affected occlusal investigation site (r_s = 0.93-0.95, P< 0.001). Concentrating on those carious occlusal sites, there was a strong to very strong relationship between the variables area, volume and percent of mineral loss within the enamel lesion (r_s = 0.75-0.82, P< 0.001). As the carious lesions became deeper there was progressive mineral loss within

the enamel, but when the lesion reached into dentine there was a dramatic increase in lesion volume and mineral loss within the overlying enamel lesion of up to 90% (none of the lesions in this study were cavitated).

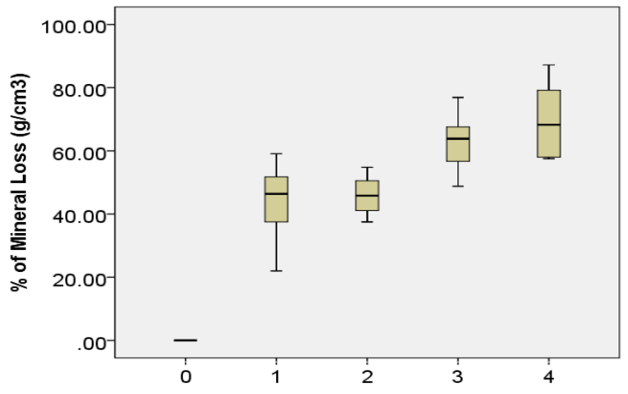
A very strong direct relationship was found between the variables area of the lesion, volume of the lesion and the percentage of mineral loss within enamel at each proximal surface (r_s = 0.97-0.99, P< 0.001). Concentrating on carious lesions only, there was a strong relationship between area and volume of the lesions (r_s = 0.72, P< 0.001). Despite this, the relationship between the area and percentage mineral loss within the enamel lesion and between the volume and the percentage mineral loss was only moderate (r_s = 0.41, P< 0.001). In addition, the percentage mineral loss in enamel for proximal lesions was generally less than that for occlusal lesions even when deeper dentine lesions were evaluated with mineral loss mainly confined to between 20 to 60 %.



Median	0	0.1	0.25	0.92	2.25
Min.	0	0.03	0.09	0.15	1.37
Max.	0	0.23	0.65	1.95	3.22

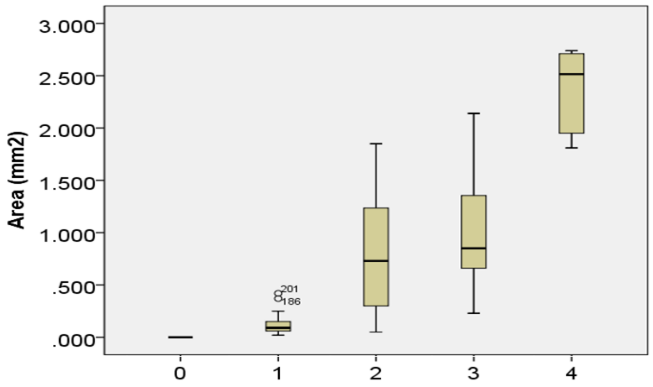


Median	0	0.047	0.056	0.127	9.13
Min.	0	0.015	0.023	0.104	4.55
Max.	0	0.103	0.096	5.79	12.31

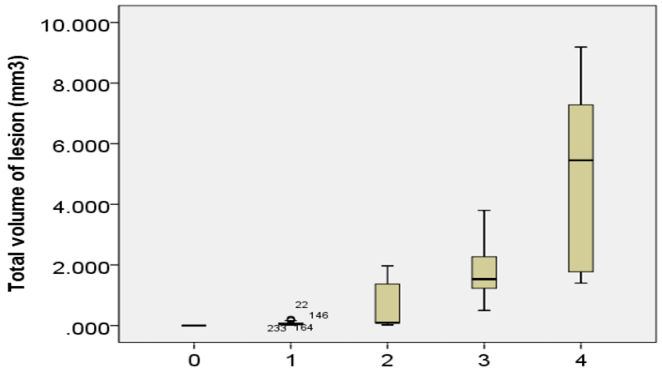


Median	0	46.4	45.8	63.8	68.3
Min.	0	22	37.5	48.8	57.5
Max.	0	59.1	54.8	76.9	87.2

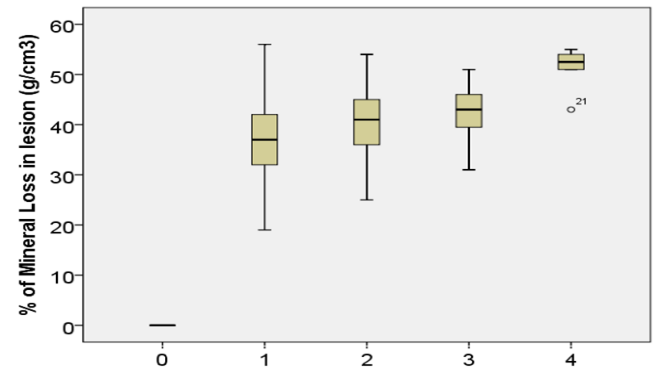
Figure 5.11 Frequency distribution (median, minimum and maximum) of the area of lesions, the volume of lesions and percentage of mineral loss in lesions (y-axis) for each Downer classification score (x-axis) for the deepest occlusal investigation site.



Median	0	0.09	0.73	0.85	2.52
Min.	0	0.02	0.05	0.23	1.81
Max.	0	0.42	1.85	2.14	2.74

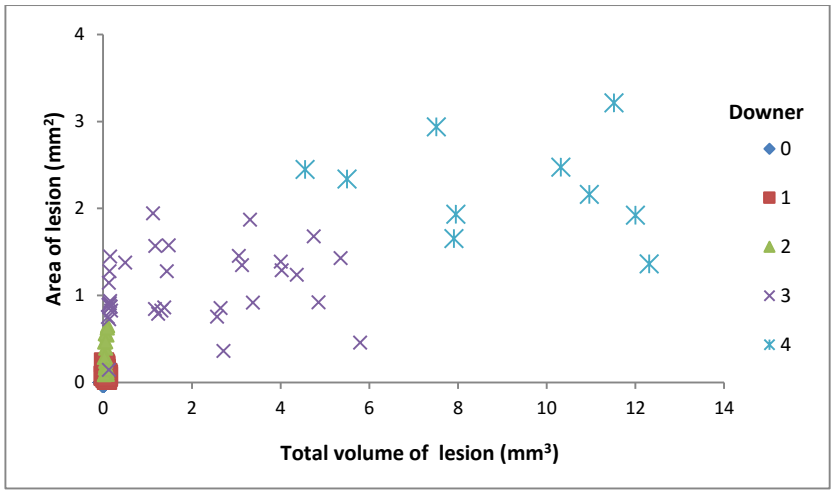


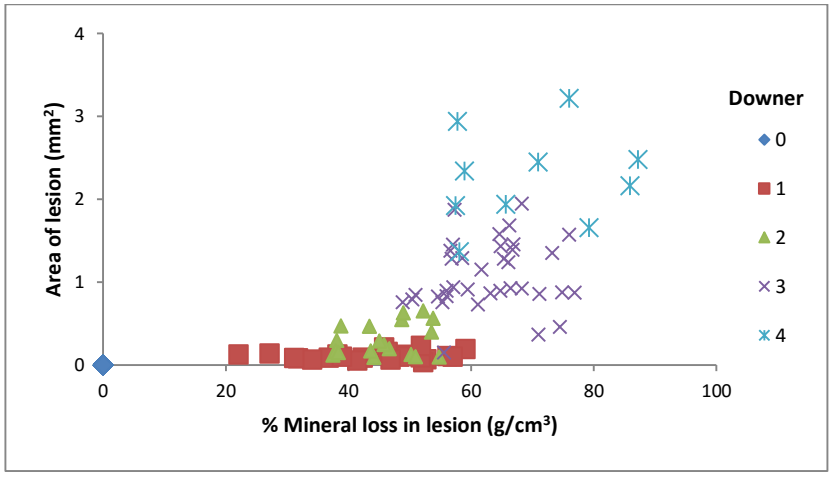
Median	0	0.043	0.094	1.53	5.45
Min.	0	0.013	0.02	0.51	1.39
Max.	0	0.223	1.97	3.8	9.19



Median	0	37.1	40.5	43.9	52.5
Min.	0	19.2	25.3	31.3	43.4
Max.	0	56.1	54.4	51.2	55.5

Figure 5.12 Frequency distribution (median, minimum and maximum) of the area of lesions, the volume of lesions and percentage of mineral loss in lesions (y-axis) for each Downer classification score (x-axis) for the proximal surfaces.





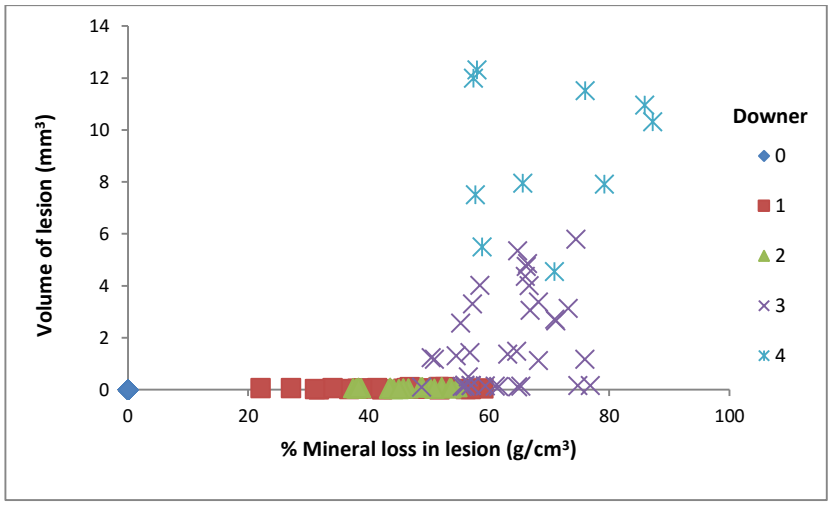
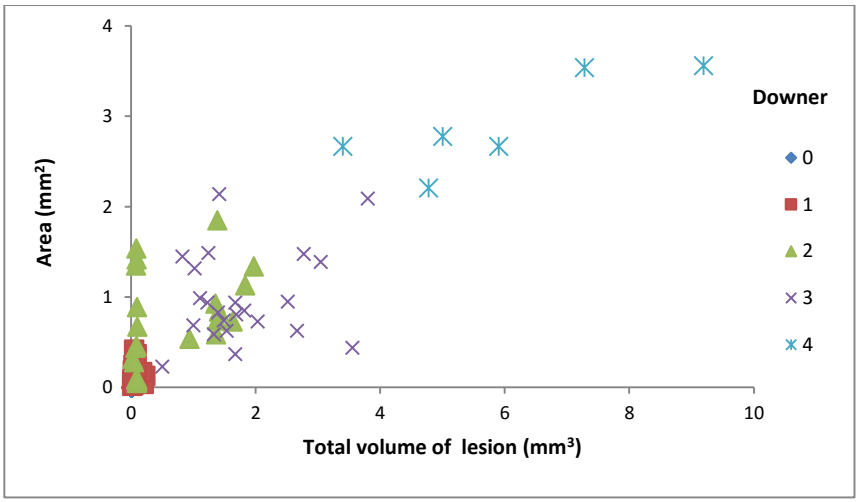
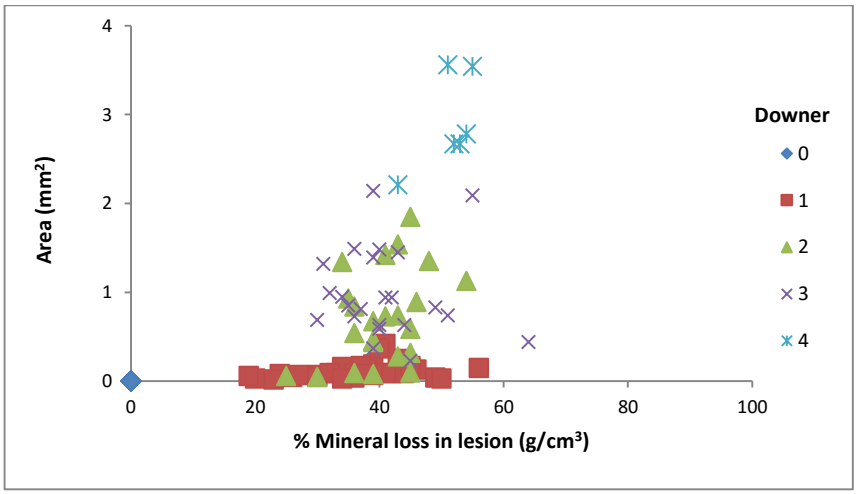


Figure 5.13 Scatterplots of the relationship between the area of lesions, volume of lesions and percentage of mineral loss at most affected occlusal investigation site according to each consensus Downer classification score.





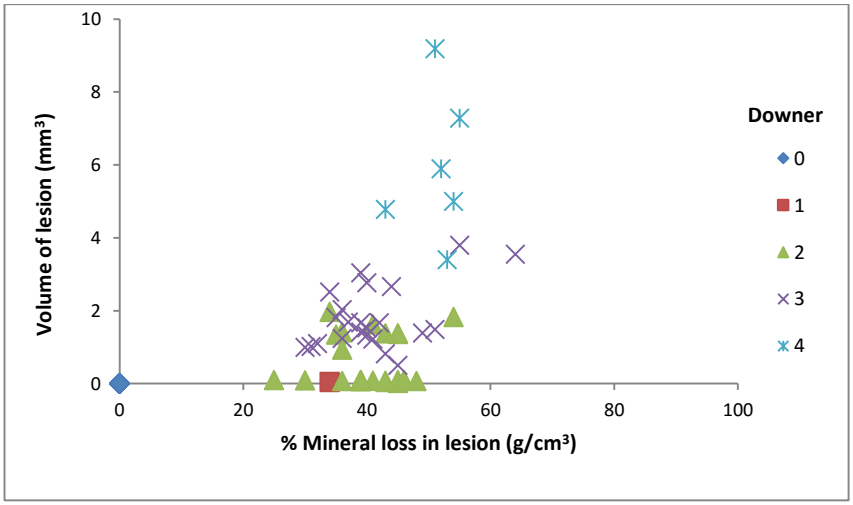


Figure 5.14 Scatterplots of the relationship between the area of lesions, volume of lesions and percentage of mineral loss at each proximal surface according to each consensus Downer classification score.

Downer classification: The Kruskal-Wallis test revealed that there was a very highly significant differences between Downer classification categories when expressed as either Micro-CT evaluation of lesion area, Micro-CT evaluation of lesion volume or Micro-CT evaluation of mineral loss in enamel. These observations were valid for the worst affected occlusal investigation sites and proximal lesions if present (Table 5.1). When statistically significant differences occurred, they were investigated further using the Dunn-Bonferroni multiple pairwise comparison test (results summarised in Table 5.2).

	Downer classification consensus scores			
	Kruskal-Wallis (H-test)			
	D.F. (n-1)	**Area (mm^2)**	**Volume (mm^3)**	**% of Mineral Loss**
Occlusal (140 teeth)	4	132.26***	132.47***	126.55***
Proximal (280 surfaces)	4	273.93***	275.20***	269.31***

Table 5.1 Kruskal-Wallis results of Downer consensus scores on the area, volume of lesion and percentage mineral loss on occlusal and proximal surfaces. ***P<0.001; N.S. Not significant; D.F. denotes for degree of freedom.

As would be expected, the area and volume of occlusal lesions confined to enamel were statistically different than those occlusal lesions extending into dentine. There was also a difference in the percentage mineral loss in enamel between enamel and dentine occlusal lesions. However, the area of proximal lesions confined to the inner half of enamel were not statistically different than lesions extended to the outer half of dentine. There was no statistically significant difference in the percentage mineral loss in enamel between proximal deep enamel lesions (Downer 2) and early enamel lesions (Downer 1) or lesions that extended to outer half of dentine.

	Consensus Micro-CT (Downer) with Area of most affected site (mm²)				
	Occlusal				
Proximal	Pairwise comparisons	1	2	3	4
	1		0.93	***	***
	2	***		***	***
	3	***	0.89		0.09
	4	***	***	0.33	

	Consensus Micro-CT (Downer) with volume of lesion (mm³)				
	Occlusal				
Proximal	Pairwise comparisons	1	2	3	4
	1		1.00	***	***
	2	0.76		***	**
	3	**	1.00		1.00
	4	***	***	0.14	

	Consensus Micro-CT (Downer) with % of mineral loss in enamel (g/cm³)				
	Occlusal				
Proximal	Pairwise comparisons	1	2	3	4
	1		1.00	***	***
	2	0.76		***	**
	3	*	1.00		1.00
	4	***	**	0.33	

Table 5.2 Dunn-Bonferroni's pairwise comparison test of the area, volume of lesion and percentage mineral loss in the enamel lesion according to Downer consensus scores on occlusal and proximal surfaces where occlusal results are in red and bold font, proximal results are black and normal font; * P<0.05; **P<0.01 and ***P<0.001. Note: This is not a cross-tabulation of occlusal versus proximal lesions.

Table 5.3 and 5.4 summarises the Kruskal-Wallis test results for differences in the area and volume of the lesion, and the percentage mineral loss in enamel between the consensus scores for all caries detection methods, including the categorical converted data for DIAGNOdent Pen and CarieScan PRO, for occlusal and proximal surfaces. Where significance was found a Dunn-Bonferroni multiple pairwise comparison test was undertaken to determine where the differences of the aforementioned parameters occurred between scores for each detection method.

	Occlusal (140 teeth)			
	Kruskal-Wallis (H-test)			
	D.F. (n-1)	**Area (mm^2)**	**Volume (mm^3)**	**% of Mineral Loss**
ICDAS	4	69.92***	71.82***	61.22***
FOTI	5	67.58***	71.66***	66.11***
BW	4*	20.73***	20.32***	15.09**
CBCT	4*	88.05***	84.21***	74.19***
PCBCT	2*	23.66***	30.26***	20.89***
DD	-	N.S.	N.S.	N.S.
CS	-	N.S.	N.S.	N.S.

Table 5.3 Kruskal-Wallis test results between consensus scores of caries detection methods and area, volume and percent of mineral loss for occlusal surface where *Missing categorical group upon consensus; **P <0.01; ***P<0.001; N.S. Not significant; D.F. denotes for degree of freedom.

It was found that there were no significant differences in the area of the lesion, volume and mineral loss in enamel according to the consensus scores of Bitewing radiographs for the proximal surfaces. In addition, there were no significant differences between those parameters according to the converted consensus categorical scores of DIAGNOdent Pen and CarieScan PRO for occlusal and proximal surfaces. Accordingly, no Dunn-Bonferroni pairwise comparison test follow up analyses were undertaken.

The Dunn-Bonferroni pairwise comparison for ICDAS is shown in Table 5.5 for illustrative purposes.

	Proximal (280 surfaces)			
	Kruskal-Wallis (H-test)			
	D.F. (n-1)	Area (mm^2)	Volume (mm^3)	% of Mineral Loss
ICDAS	4	113.73***	121.48***	117.74***
FOTI	5	76.36***	87.07***	77.14***
BW	-	N.S.	N.S.	N.S.
CBCT	5	128.12***	124.89***	104.53***
PCBCT	5	20.03***	18.68***	18.19***
DD	-	N.S.	N.S.	N.S.

Table 5.4 Kruskal-Wallis test results between consensus scores of caries detection methods and area, volume and percent of mineral loss for proximal surface where; ***P<0.001; N.S. Not significant; D.F. denotes for degree of freedom.

Regarding ICDAS consensus scores for occlusal surfaces, there was no significant difference in the area and volume of lesions scored with 2, 3 and 4. There was a significant difference in the area and volume of the lesion and percentage mineral loss in enamel of occlusal surfaces/lesions scored 0 and 1, 2, 3, and 4. Of relevance, there was also significant difference in area and volume of the occlusal lesions between ICDAS scores 1 and 2. However, the only significant difference in the area and volume of proximal lesions and percent mineral loss in enamel was between surfaces/lesions scored with zero and lesions scored with 1, 2, 3 and 4.

Regarding FOTI consensus scores for occlusal surfaces, the only significant difference found in the area, volume of lesions and percent of mineral loss was between lesions scored with 0 and 1, 2, 3 and 4; and lesions scored with 1 and 3. However, for proximal lesions the only significant difference found in the area and volume and percent mineral

loss in enamel was between surfaces/lesions scored with 0 and lesions scored with 1, 2, 3 and 4.

	Consensus ICDAS with Area of most affected site (mm^2)					
	Occlusal					
	Pairwise comparisons	0	1	2	3	4
Proximal	0		**	***	***	***
	1	**		*	**	**
	2	***	1.00		1.00	1.00
	3	***	1.00	1.00		1.00
	4	***	0.88	1.00	1.00	

	Consensus ICDAS with volume of lesion (mm^3)					
	Occlusal					
	Pairwise comparisons	0	1	2	3	4
Proximal	0		**	***	***	***
	1	**		*	*	**
	2	***	1.00		1.00	1.00
	3	***	1.00	1.00		1.00
	4	***	0.34	1.00	1.00	

	Consensus ICDAS with % of mineral loss (g/cm^3)					
	Occlusal					
	Pairwise comparisons	0	1	2	3	4
Proximal	0		**	***	***	***
	1	**		0.36	0.08	0.22
	2	***	1.00		1.00	1.00
	3	***	1.00	1.00		1.00
	4	***	0.72	1.00	1.00	

Table 5.5 Dunn- Bonferroni's pairwise comparison test of the area, volume of lesion and % of mineral loss in enamel according to ICDAS consensus scores on occlusal and proximal surfaces where occlusal results are in red and bold font, proximal results are black and normal font; * P<0.05; **P<0.01 and ***P<0.001. Note: This is not a cross-tabulation of occlusal versus proximal lesions.

Regarding Bitewing radiographs consensus scores for occlusal surfaces, the only significant difference was found in the area and volume of lesions and percent of mineral loss in enamel which was between surfaces/lesions scored with 0 and lesions scored with 3. Also of note is that the consensus score of 1 was not recorded in the entire sample.

Regarding CBCT consensus scores for occlusal surfaces, the only significant difference was found in the area, volume of lesions and percent of mineral loss which was between lesions scored with 0 and lesions scored with 1, 2, 3, 4 and 5. For proximal surfaces there was a significant difference in the area between score 0 and 1, 2, 3, 4 and 5. For Volume there was only a significant difference between scores 0 and 2, 3, 4 and 5 and finally for percentage mineral loss there was only a significant difference between scores 0 and 2, 3, and 4. Also of note is that the consensus score of 1 was not recorded in the entire sample.

Regarding PCBCT consensus scores for occlusal surfaces, the only significant difference was found in the area, volume of lesions and percent of mineral loss which was between surfaces/lesions scored with 0 and lesions scored with 3 and 4. Also of note is that the consensus score of 1 and 2 were not recorded on the occlusal surface in the entire sample. For proximal surfaces the only significant difference in the area, volume of proximal lesions and percent of mineral loss in enamel was between surfaces/lesions scored with 0 and lesions scored with 2.

There was a ‘very weak’, to ‘weak’ direct relationship between the consensus reading from the raw data for **DIAGNOdent Pen** and **CarieScan PRO** at the deepest occlusal investigation sites and proximal surfaces with the area, volume of the deepest lesion and the percentage mineral loss (r_s = 0.07-0.26, $P < 0.01$).

5.4 Discussion

Micro-CT is an imaging system that enables three dimensional volumetric assessment of the internal structures of objects. Initially, it was used to acquire three dimensional images from which lesion depth could be estimated based on a subjective visual assessment of the image of the lesion (Mitropoulos *et al.*, 2010; Kawato *et al.*, 2009; Djomehri *et al.*, 2015). In the last few years, there has been a shift towards extracting key parameters from these images such as grayscale values, LAC and mineral content, through the quantitative analysis of the tissue under investigation (Swain and Xue, 2009; Maire and Withers, 2014). Since this method is non-destructive, it can be used in longitudinal studies as the same object can be tested many times under various conditions (Davis and Wong, 1996). In addition, retention of tooth structure enables the possibility that the teeth can be used again in further studies. In the last few years this method has been utilized to explore the dynamic process of caries in enamel (Huang *et al.*, 2007; Wong *et al.*, 2004) and dentine (Wong *et al.*, 2006; Willmott *et al.*, 2007) as well as the effectiveness of caries removal techniques (Neves *et al.*, 2011).

Although Micro-CT has proved to be a valuable method for determining lesion depth by using different subjective criteria, no attempt has been carried out to explore and quantify the natural three dimensional complex structure of caries by utilising the inherent features of Micro-CT.

There are a number of potential problems associated with polychromatic cone beam Micro-CT systems and these include ring artefacts, beam hardening and low signal to noise ratio. However, special reconstruction algorithms can significantly reduce some of these effects (Davis and Elliott, 1997) to a degree that they are thought to be inconsequential (Davis and Elliott, 2003). To reduce the effect of beam hardening in this

study, the μCT 40 Scanco system was installed with a 0.5 mm Aluminium filter in the beam path for the selective removal of low energy x-rays. In addition, the manufacturer implements a sophisticated beam hardening correction algorithm in their systems which are applied during the construction of images.

The Scanco solid calibration phantom consists of different densities of HA crystals ranging from zero to 800 mg/cm^3 embedded in epoxy resin to mimic the surrounding soft tissues and fitted on a mounting rod. However, for an accurate quantitative use of Micro-CT systems, external means of calibration is recommended to overcome the problem associated with the variations associated with the automated internal calibration within the software of the machine (Schwass *et al.*, 2009). As such, a sintered hydroxyapatite disk was placed on the top of each specimen for standardization of Micro-CT scanning procedure to avoid any systematic errors which can arise from potential x-ray spectrum shift between scans.

The method developed in this study for volumetric analysis of the lesions was based on selecting an appropriate threshold of greyscale values from the reconstructed images of the enamel, dentine and the background, so called "segmentation". Segmentation is the procedure by which regions in the images belonging to different grayscale values are defined and selected by simple thresholding to create a binary image (Maire and Withers, 2014; Carrera *et al.*, 2015). To further improve lesion identification and selection of appropriate threshold for enamel and dentine, the contrast was set at 20% and the noise in the images was reduced by applying a median filter of one pixel radius. Neves *et al* (2011), in a study investigating the effectiveness of modern caries excavation techniques using Micro-CT images imported into Image J, used a median filter of 7-pixel radius to

preserve edge sharpness. This process also facilitates the segmentation of the dental hard tissues based on their grayscale values (Taylor *et al.*, 2010).

To set the thresholds for the process of segmentation, a slice representing sound enamel and dentine was chosen to perform thresholding. This was done in two stages: enamel threshold and dentine threshold as both tissues have different grayscale values and mineral concentration (Djomehri *et al.*, 2015; Davis and Wong, 1996). Both stages are described in details in section 5.2.3.

Partial volume effects are present on any digital image whether 2D or 3D. The effect is seen as pixels on the edges of an object including grayscale values representing both the object and background. The signal in these pixels is therefore the area weighted mean of the object and background and this can be seen in the images as a fine dark line. The partial volume effect makes it harder to select the appropriate threshold for a specific tissue, especially on small or thin objects (Hoffman *et al.*, 1979). As such, if the edges were included, the volume measures would be a slight overestimation and the mineral density a slight underestimation (Farah *et al.*, 2010; Davis *et al.*, 2013) for this reason, in this study, the pixels representing the edges of tissues during thresholding were not included.

As caries is a natural dynamic process of remineralisation and demineralisation, in its early stages, the lesion exhibits a complex three dimensional uneven structure guided by enamel prism direction on occlusal fissures and smooth proximal surfaces (Kidd and Fejerskov, 2004). As such, it was more valuable to explore the lesion in terms of area (2D) and volume (3D) using Micro-CT. In this study, the manipulation of the 2D tomographic slices using Photoshop CS6 was a beneficial method to determine the area of the lesion at the deepest aspect on occlusal and proximal surfaces. In the same way,

the stack of the tomographic slices representing the beginning and the end of the lesion at a slice thickness of 0.1mm together with the modified Photoshop script facilitated the measurement of the volume of the lesion (enamel and dentine).

The MuCAT2 machine developed at Queen Mary, University of London was capable of determining the density of the hydroxyapatite disk in an accurate way. This machine was tested by Davis *et al,* (2013) to demonstrate the ability to provide high quality tomographic images and hence an accurate estimation of the density of the hydroxyapatite disk. Davis et al., (2013) found that the quantification accuracy in determining the density of the HA disk was better than 1%.

Enamel is a highly mineralised structure containing 95% wt. inorganic substance which could vary between the inner and outer enamel where the lowermost concentration is near the EDJ and CEJ (Weatherell *et al.*, 1974; Anderson *et al.*, 1996; Wong *et al.*, 2004; Zou *et al.*, 2009). This study adopted methods for calculation of the mineral density of enamel that have been used in previous studies which ignored the attribution of the inorganic component and assumed that the mineral content of enamel was pure HA, the density of which is 3.15 g/cm3 (Wong *et al.*, 2004; Willmott *et al.*, 2007; Cochrane *et al.*, 2012).

Inaccuracies in the estimation of mineral densities as a result of the beam hardening effects can be solved through mineral density calibration by using a series of phantoms of different concentrations to create a direct relationship between the grayscale values and mineral density (Farah *et al.*, 2010; Huang *et al.*, 2007; Zou *et al.*, 2009). Such material should be homogeneous and possess x-ray attenuation properties which reflect the composition of the scanned specimen (Schweizer *et al.*, 2007). HA phantoms have been used frequently to determine the mineral concentration of enamel because such material is homogenous and suitable to determine the highest and lowest range of enamel

mineral content as well as being the major constituent of bone and teeth (Lai *et al.*, 2014; Schwass *et al.*, 2009; Huang *et al.*, 2007; Neves *et al.*, 2011).

In this study, a novel method was developed to measure the mineral concentration of the whole enamel part of the lesion. The percentage mineral loss was used in this study to represent the mineral concentration of the enamel in the affected lesion at the deepest aspect of the lesion and expressed as percentage of sound enamel. This method will facilitate the comparison between lesions irrespective of the variation of the mineral concentration in enamel among teeth and within teeth i.e. under the cusp, near the fissure and the surface of the tooth (Wong *et al.*, 2004; Clementino-Luedemann and Kunzelmann, 2006; Anderson *et al.*, 1996).

After consideration of previous studies, a lesion in this study was defined when the mineral loss was between 20% and 95% of sound enamel. Ten Cate *et al*, (1996) explored the reproducibility of artificial enamel lesion analysis under four different laboratory conditions using transverse microradiography. He defined the onset of the lesion either as zero or 20 % of mineral content and the start of the sound enamel as 80, 95 or 100% of the sound enamel. It was found that changes in the definition of the onset of the lesion had a great impact on the calculation of the integrated mineral loss. In addition, Cochrane *et al.*, (2012) and Hamba *et al.*, (2012) measured the lesion depth at the deepest aspect of natural enamel lesions and in both studies the lesion was defined as the points where the mineral concentration was 20% i.e. onset of caries and 95% i.e. end of caries of sound enamel.

The mineral loss in the enamel part of the lesion only was considered in this study, even if the lesion extended into dentine. Excluding the affected dentine mineral concentration was done for two reasons, the first was because determining the mineral concentration of

dentine remains a major challenge as calibration of Micro-CT device by a representative material is very difficult (Zou *et al.*, 2009) because dentine is a complex composite structure which is composed of 70% wt. inorganic material, 20% wt. organic matrix and 10% wt. water (Frank, 1999; Marshall Jr *et al.*, 1997). Secondly, the mineral content of normal dentine is characterised by heterogeneity in the distribution of the mineral concentration within and around the dentinal tubules (Swain and Xue, 2009). Thus it is difficult to develop a homogenous material of similar composition to dentine to achieve a linear relationship between the grayscale values and the mineral density of dentine (Schwass *et al.*, 2009; Willmott *et al.*, 2007; Zou *et al.*, 2009).

For comparison with the results of previous studies, the mean mineral concentration of sound enamel in this study was 2.72 g/cm^3 with a range between 2.88 g/cm^3 and 2.33 g/cm^3. The mean mineral concentration of enamel in the affected lesion was 1.36 g/cm^3 with a range between 0.9 g/cm^3 and 2.12 g/cm^3. These findings are comparable with the results of previous Micro-CT studies (Table 5.6) which have looked at the distribution mineral concentration in human teeth (Clementino-Luedemann and Kunzelmann, 2006; Anderson *et al.*, 1996; Wong *et al.*, 2004); mineral concentration in artificial and natural enamel lesions (Cochrane *et al.*, 2012; Huang *et al.*, 2007; Dowker *et al.*, 2003; Dowker *et al.*, 2004); the mineral concentration in enamel with defects such as hypo-mineralization (Farah *et al.*, 2010; Fearne *et al.*, 1994) and mineral concentration in enamel after application of caries removal techniques (Ahmed *et al.*, 2012; Neves *et al.*, 2011). A summary of the findings of the relevant studies investigating the mineral concentration of sound enamel and carious enamel lesions is presented in Table 5.6.

This summary shows that all studies measuring the mineral concentration of sound enamel or affected enamel were defined as the average of different regions of interest

(ROI), of different sizes and at various locations within the enamel or the lesions. These ROI were drawn on cross-sections or sagittal sections through the teeth under investigation, whereas in this study the average mineral concentration of the entire enamel lesion within the tomographic slice was calculated. The observed variations in the results of mineral densities of sound and carious enamel determined by Micro-CT studies could be attributed to the different commercially available Micro-CT machines available in the market i.e. different settings, sensor characteristics and different sizes of samples, wide range of calibration modalities and different analytic methods for measurement of mineral concentration (Lai *et al.*, 2014).

The large variation in the shapes and sizes of the enamel lesions observed in this study and the lesion mineral loss/content was consistent with the findings of previous studies which investigated the characteristics of natural and artificial white spot lesions (Huang *et al.*, 2007; Cochrane *et al.*, 2012; Dowker *et al.*, 2003). However, this is the first study to evaluate such a large number of teeth and describe the lesions based on their subjective lesion depth.

The relation between the area, volume of occlusal lesions and the mineral loss in the affected enamel demonstrated that as the occlusal lesions go deeper into dentine, the area and consequently the volume of the lesion increased dramatically especially for lesions with Downer score 3 and 4. However, the percentage mineral loss within the enamel part of the lesion showed a steady increase from small, enamel lesions to more extensive lesions extending beyond the EDJ when the percentage mineral loss for the enamel part can reach up to 90% of sound enamel. Contrary to this finding for occlusal lesions, the proximal lesions behaved in a different way.

Study	MD OF SOUND ENAMEL	MD of enamel lesion	Type of teeth
(Cochrane *et al.*, 2012)	2.57-3.10	2.48-1.73	Line profile was drawn through the lesion at its deepest point and perpendicular to the surface. 12 molar teeth
(Ahmed *et al.*, 2012)	2.3-2.8		21 primary molars hemi-sectioned through the open carious cavities
(Farah *et al.*, 2010)	2.37-2.62		10 sound molars and 10 hypo-mineralised Permanent molar teeth in horizontal sections
(Neves *et al.*, 2011)	2.89		One molar tooth with dentine caries
(Huang *et al.*, 2007)	2.65-2.89	2.58-1.43	Horizontal sections of four permanent molars, areas of ROI of three pixels from enamel surface till EDJ
(Clementino-Luedemann and Kunzelmann, 2006)	2.7-2.8		Sagittal enamel section of five permanent molar teeth
(Dowker *et al.*, 2004)	2.7-2.8		Sagittal enamel section of one premolar tooth with proximal lesion
(Wong *et al.*, 2004)	2.81		Sagittal section of 11 deciduous molars
(Dowker *et al.*, 2003)	2.6-3.1	1.8-0.9	Three whole molars
(Anderson *et al.*, 1996)	2.82-2.65		ROI in cross-sectional of two Premolars
(Fearne *et al.*, 1994)	2.60-2.78		Different ROI in longitudinal sections OF One primary molar

Table 5.6 Relevant Micro-CT studies investigating the mineral concentration of sound and carious enamel in permanent and primary teeth.

For proximal lesions, the area and volume of the deep enamel lesions are comparable with those lesions which extended into the outer half of the dentine. This could be explained by the direction of the enamel prisms on the proximal surfaces where the lesions assume the shape of a triangle with the base towards to the surface, so the lesions extending into

dentine, and recorded as a Downer score 3, could be a lesion which has just extended to dentine by a small area beyond the EDJ. This was the opposite for an occlusal lesion where the shape of caries is conical with the base is towards the EDJ. In this case as the lesion progresses, it will spread laterally through EDJ and later deep into dentine (Kidd and Fejerskov, 2004; Wong *et al.*, 2006; Kidd and Fejerskov, 2013; Bjørndal and Thylstrup, 1995). In addition, on the proximal surface the percentage mineral loss in the enamel lesion reached only up to 60% of sound enamel, even when the lesions extended much deeper into dentine. This could be attributed to the fact that the proximal smooth surfaces are more readily exposed to saliva where the direct effect of fluoride on such surfaces is more profound than for occlusal surfaces with complex morphology featuring deep fissures causing difficulty of plaque removal (Rodrigues *et al.*, 2008). In a systematic review performed by Pitts (1983) and supported by a further study by Mejàre *et al* (1999) on the incidence and progression of proximal caries, they concluded that progression of early natural enamel lesions which occurred on proximal surfaces was a slower process and remained unchanged for longer periods of time compared with occlusal caries (Zandoná and Zero, 2006).

The Downer classification system was able to differentiate between lesions when expressed as lesion area, volume and percentage mineral loss. Further analysis, revealed that **occlusal lesions** confined to enamel and lesions extending into dentine were different in terms of area, volume and percentage mineral loss within the enamel. This would mean that using the EDJ as a threshold in the classification was able to distinguish between enamel and dentinal lesions based on their shapes, sizes and mineral concentration. Using this information, different diagnostic and treatment strategies can be applied to such discrete categories. However, for **proximal lesions**, as mentioned above, deep enamel

and early dentinal lesions were not different in their shapes and sizes and their mineral content within the enamel. This would mean again that proximal lesions behave in a different way than occlusal lesions and the EDJ is not a suitable threshold for differentiating enamel and dentinal lesions based upon caries severity. Using another classification system such as the ERK classification system would be more beneficial to differentiate between enamel lesions and early dentinal lesions where the middle third of dentine is the threshold for applying the operative intervention.

There was good agreement between examiners for all detection methods (with the exception of the CarieScan PRO) in order to achieve a consensus score for the worst affected occlusal site and proximal surface. This would mean that the examiners had adequate clinical experience and a good clinical background to achieve a consensus score for each surface. Regarding consensus occlusal scores for radiographic methods, examiners failed in most instances to record a score 1 and 2, in other words, they couldn't agree on detecting enamel lesions. This highlights again the difficulty of diagnosing lesions confined to enamel from radiographs because of the superimposed sound buccal and lingual enamel in the images (Krzyzostaniak *et al.*, 2014; Wenzel, 1995; Wenzel, 2004a) and the complexity of occlusal anatomy (Wenzel *et al.*, 1991a).

The relationship between consensus occlusal scores for ICDAS and the area, volume and mineral content in the lesion was evaluated. It was obvious that there was a difference in the area and volume between lesions scored 1 and 2 on the ICDAS system. Based upon this it would seem relevant to still retain the scores 1 and 2 in the system rather than collapsing them into one score as suggested in the ICCMS documentation (Pitts and Ekstrand, 2013). In relation to ICDAS it would appear that the mineral loss is not a suitable sole evaluation parameter to distinguish between lesions severity scores.

However, this is not the case regarding proximal lesions, where the classification couldn't distinguish between enamel and dentine lesions based on the size and percentage mineral loss within the enamel. This could be explained by the low prevalence of caries in proximal surfaces (70%), that is, there were not enough lesions in each ICDAS category to detect differences statistically and to reflect the true relationship between mineral loss and ICDAS scores.

Regarding FOTI consensus scores for the occlusal surface, the only difference in the lesions area, volume and mineral loss was found between early enamel lesions (score 1) and early dentine lesions (score 3). This would mean that FOTI classification system used in this study couldn't distinguish between enamel and dentine lesions when the D_3 threshold was set at FOTI scores between 2 and 3, where deep enamel lesions cannot be differentiated from those that extended into dentine. Modification of the FOTI classification criteria would therefore be required based on these results to be able it to distinguish between enamel and dentine lesions. For example, combining the detection of enamel lesions in one category and dentine in another category. Using the FOTI classification on proximal surfaces revealed poorer results with no significant differences in area, volume and mineral content could be found between each FOTI caries code.

Regarding the bitewing (BW) radiograph consensus scores, the only difference in area, volume and mineral loss was between sound and dentine lesions only. This would be expected due to the lack of achieving a consensus score for enamel lesions (codes 1 and 2). This could be explained again by the difficulty of diagnosing enamel lesions on the occlusal surface due to the bulk of sound tooth structure buccally and lingually attenuating the x-ray beam (Kidd *et al.*, 2003a) and the amount of mineral loss required for the lesions to be seen radiographically which is about 30-40% (Wenzel, 2004b). However, it was a

bizarre finding that consensus scores of BW on proximal surface failed to differentiate between lesions and sound tooth structure.

The same issue existed with the relationship between CBCT and Panoramic view (2D image) of CBCT consensus scores for occlusal surface evaluation and the area, volume and mineral loss in the lesions. It was obvious that the classification failed to distinguish between enamel and dentine lesions. It seems that the problem of detection of enamel lesions on occlusal surfaces is still a difficult task despite the fact that the images are three dimensions, of better quality and at higher resolution (Ertaş *et al.*, 2014; Krzyzostaniak *et al.*, 2014; Young *et al.*, 2009). However, for the proximal surfaces the issue was worse than was expected as the consensus scores achieved were not able to differentiate between sound and early enamel lesions. This meant that this method was not sensitive enough to detect enamel lesions in terms of early changes in the mineral content and consequently the formation of the lesion. In addition, PCBCT was not able to distinguish between sound and dentine lesions.

When the DIAGNOdent Pen readings were converted into categorical data according to Lussi and Hellwig (2006) and for the CarieScan PRO according to the manufacturer's instructions, no difference in lesion area, volume or mineral loss could be found between each of the categories. This would mean that interpreting caries lesion severity based upon these parameters is problematical both on the occlusal and proximal surfaces. It could be that the thresholds set for converting the readings into categorical data are inappropriate and further work on thresholding could reveal better relationships, however, this is unlikely due to the range and prevalence of lesions in this study. Another reason was mentioned by Ástvaldsdóttir *et al.*, (2010) regarding fluorescence, it is believed to originate from bacteria or their metabolites. Hence, there is a poor correlation between

LF readings and the mineral content, but possibly better correlation with the presence of infected dentin.

5.5 Conclusions

Within the limitations of the present study, the conclusions that could be drawn from this study were:

1. The agreement between the eleven examiners enabled consensus scores to be reached for each caries detection method for occlusal and proximal surfaces of 140 teeth with the exception of the CarieScan PRO which had the least agreement of only 50% on the occlusal surfaces.
2. A new method was devised for area measurement and volumetric analysis of the caries lesions in three dimensions using Micro-CT. In addition, a novel method was developed for the measurement of mineral content within carious lesions.
3. The relationship between the variables area, volume and mineral loss in the occlusal lesion were stronger than that for proximal lesions. Moreover, the percentage mineral loss in enamel for deep proximal lesions was generally less than that for occlusal deep lesions.
4. There were highly significant differences in the area, volume and percentage mineral loss between enamel and dentine lesions on the occlusal surfaces according to Downer classification system whereas the behaviour of proximal lesions was different. Those proximal deep enamel lesions (Downer 2) were not different in shape and mineral loss compared with proximal lesions which extended halfway to the pulp (Downer 3).

5. There was a significant difference in the area, volume and percent of mineral loss for occlusal and proximal lesions according to the consensus scores for ICDAS, FOTI and CBCT and PCBCT. However, there were no differences in these variables when evaluating the consensus scores of the categorical data for DIAGNOdent Pen and CarieScan PRO on occlusal surfaces and for BW consensus scores on proximal surfaces.
6. The differences between the categories of each classification for each caries detection method were different from each other in terms of area, volume and percentage mineral loss in enamel for occlusal and proximal surface lesions. However, the differences were less obvious on the proximal surface due to low caries prevalence.

CHAPTER SIX

Summary of Conclusions and Further Suggestions

This thesis was based on a laboratory study which was carried out under ideal and standard conditions. The sample consisted of mainly premolar and third molar teeth, as these were the only non-cavitated teeth routinely extracted. As such the third molar teeth may be morphologically different to other molars, and have higher caries prevalence due to patients' inability to maintain the occlusal surface plaque free. It may also be argued that the sample size and the number of examiners recruited in this study was higher than in many other studies giving a greater strength to the results and conclusions. The teeth were extracted before 2006, stored in water under room temperature.

The *in vitro* model used in this study was designed to mimic that in the real clinical situation that is setting teeth in arches and all the examinations carried out in phantom heads. This study had several limitations though firstly, it was performed *in vitro* under standard conditions of clean teeth, secondly, the teeth included in the study had no restorations or defects and thirdly, no soft tissue was used in the radiographic techniques. Therefore, the results of this study should be extrapolated to clinical settings with caution and may indeed reflect a superior performance than would be found clinically on patients.

6.1 Summary of Conclusions:

Within the limitations of this study, a summary of the answers to the research questions posed in previous Chapters are presented in the following sections:

The performance in terms of reproducibility of conventional and novel detection methods for occlusal and proximal caries were as follows:

1. The inter- and intra-examiner reproducibility of the DIAGNOdent Pen was superior compared with other examination methods for occlusal caries detection

and showed strong agreement between and within examiners. The CarieScan PRO was inferior to other examination methods for occlusal caries detection and had poor agreement between and within examiners.

2. The inter- and intra-examiner reproducibility of the ICDAS was superior compared with other examination methods for proximal caries detection having substantial agreement between and within examiners. The DIAGNOdent Pen was inferior to other examination methods for proximal caries detection showing poor agreement between and within examiners.

After dichotomisation of the data to determine the reproducibility at the D_1 and D_3 diagnostic thresholds, which were used later to determine the diagnostic accuracy for each method for occlusal and proximal caries detection, the findings were as follows:

1. At D_1 diagnostic threshold the DIAGNOdent Pen was superior to other examination methods and showed a strong agreement between examiners. At the D_3 diagnostic threshold ICDAS was superior to other examination methods, showing substantial agreement between examiners. The CarieScan PRO led to the poorest reproducibility at the D_1 diagnostic threshold and the DIAGNOdent Pen had the poorest reproducibility at the D_3 diagnostic threshold for occlusal caries detection.

2. At D_1 and D_3 diagnostic threshold for proximal caries detection, ICDAS was superior to other examination methods showing substantial agreement between examiners. The DIAGNOdent Pen had the poorest reproducibility at D_1 diagnostic threshold and the Panoramic CBCT had the poorest at the D_3 diagnostic threshold.

For accurate and reliable comparison of conventional histology and Micro-CT for validation of caries detection methods. A precise and reliable locating technique was developed to accurately link occlusal investigation sites to histological section and corresponding Micro-CT image. Fabrication of custom formed system based on three dimensional prototyping to accommodate the specimen allowed Micro-CT images to be obtained in the correct plane for comparison with that of histological sections. Comparison of corresponding Micro-CT images and histological sections showed:

1. The correlation between Micro-CT and histology was very strong indicating a strong relationship for the identification of occlusal and proximal caries lesion severity using Downer and ERK classification systems as a "gold standard".
2. The high diagnostic performance of Micro-CT achieved, when compared with histology as the most accurate and conventional "gold standard" at D_1 and D_3 diagnostic thresholds, would suggest that Micro-CT has the potential to be used as an alternative to conventional histology for occlusal and proximal caries detection.

Based on the previous conclusions, the diagnostic accuracy of conventional and novel caries detection methods were determined with both histology and Micro-CT:

1. The performance of all detection methods for occlusal and proximal caries detection in terms of sensitivity and specificity was comparable when using either histology or Micro-CT as validation method at both D_1 and D_3 diagnostic threshold. This indicates that the use of Micro-CT is a reliable and alternative non-destructive method compared with histology for validation of caries detection methods.

2. For occlusal caries detection, ICDAS and CarieScan PRO were superior at D_1 diagnostic threshold whereas CBCT and ICDAS were superior at the D_3 diagnostic threshold in terms of sensitivity. In relation to specificity all techniques with the exception of the CarieScan PRO at the D_1 diagnostic threshold, produced clinically satisfactory results.
3. For proximal caries detection, ICDAS and CBCT sensitivity results were superior at both diagnostic thresholds. Specificity values for all detection methods were clinically acceptable with the exception of digital BW radiographs.
4. The performance of DIAGNOdent Pen and digital BW radiographs were poor in terms of sensitivity at both diagnostic thresholds for occlusal and proximal caries detection.

Although Micro-CT is a valuable method for analysing lesion depth by investigating individual tomographic cuts according to specific subjective criteria, it has the potential to quantitatively assess the mineral densities of dental hard tissues through attenuation of x-ray photons. In addition it could be more useful to explore the area and volume of a lesion in relation to the surrounding structures.

A new method was devised for area measurement from each Micro-CT tomographic slice and volumetric analysis of the caries lesions in three dimensions. In addition, a novel method was developed for the measurement of mineral content within carious lesions. The findings were as follows:

1. The relationship between the variables area, volume and mineral loss in the occlusal lesion were stronger than that for proximal lesions. Moreover, the percentage mineral loss in enamel for deep proximal lesions was generally less than that for occlusal deep lesions.

2. There were highly significant differences in the area, volume and percentage mineral loss between enamel and dentine lesions on the occlusal surfaces according to Downer classification system whereas the behaviour of proximal lesions was different.
3. There was a significant difference in the area, volume and percent of mineral loss for occlusal and proximal lesions according to the consensus scores for ICDAS, FOTI and CBCT and PCBCT. However, there were no differences in these variables when evaluating the consensus scores of the categorical data for DIAGNOdent Pen and CarieScan PRO on occlusal surfaces and for BW consensus scores on proximal surfaces.
4. The differences between the categories of each classification for each caries detection method were different from each other in terms of area, volume and percentage mineral loss in enamel for occlusal and proximal surface lesions. However, the differences were less obvious on the proximal surface due to low caries prevalence.

On balance it would seem that based upon reproducibility and diagnostic accuracy ICDAS has the greatest potential of all detection methods for clinicians to use in clinical practice. If this is used the dilemma of "hidden caries", caries missed from a visual examination but deep enough to be detected radiographically, is not an issue and does not exist. In fact use of the BW radiograph could lead to problems associated with false positive decisions especially on the proximal surface. The novel detection methods that give objective readings (DIAGNOdent Pen and CarieScan PRO) suffer from problems of poor diagnostic accuracy and reproducibility and should not be used alone in making clinical judgements and in fact their use as an adjunct over and above ICDAS is

questioned. A surprising result was that obtained for CBCT which performed well in comparison to other novel detection methods, and whilst it should not be used solely for the purpose of caries detection, if it is justified for any other clinical reason, it is incumbent on the requesting clinician to report on dental caries.

This thesis has clearly shown the value of Micro-CT in the evaluation and validation of caries and should be considered as the "gold standard" for further laboratory studies on caries detection devises that may be devised and used in the future. This would allow a valuable sample of teeth to be used for further research in the future and would allow validation based upon the mineral loss within lesions and three dimensional spread and destruction caused by the carious process rather than a simple two dimensional depth analysis.

6.2 Further Suggestions:

1. To further validate the results achieved in this study for clinical use, an *ex vivo* model could be considered for teeth planned for extraction, this would allow the *in vivo* and *in vitro* evaluations to be compared and the *in vitro* validity to be assessed.
2. To further mimic real situation and reduce the errors associated with radiographs, setting teeth in human dry skulls and to be covered with synthetic soft tissue equivalent material would further help to attenuate the x-rays as should be with soft oral tissues.
3. Determination and standardization of the optimum cut-off points for methods providing continuous objective measurements by using ROC analysis may help in determining thresholds for monitoring lesions over time.

4. Longitudinal studies on such devices could be carried out on artificial caries and in clinical trials to determine the ability of detection techniques for monitoring lesions.
5. Inclusion of teeth with restorations and some developmental defects that could be encountered clinically could be included to determine how the detection methods perform in detecting lesions adjacent to restorations of different materials and to determine whether the detection methods can differentiate between caries and hypoplasia of developmental origin.
6. The ICDAS new ‘wardrobe’ concept and lesion assessment criteria can be used with some of the technology-based caries detection methods currently available. The combination of the ICDAS with a technology-based method has the potential to bring forward the best characteristics of each method for enhanced caries detection and monitoring.
7. To further explore the benefits of using Micro-CT quantitative parameters in this study that is area, volume and mineral loss. A sample with high caries prevalence and even distribution of lesions with different severities would help to confirm whether differences in these criteria exist between detection classifications.

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Appendices

Appendix 2.1

NHS Tayside

22 June 2011

Professor D Ricketts
University of Dundee
Dundee Dental School
Park Place
Dundee
DD1 4HR

Dear Professor Ricketts,

R & D MANAGEMENT APPROVAL - TAYSIDE

Title: Diagnostic accuracy and reproducibility of novel caries diagnostic techniques as Determined by histology and micro-CT; a study in-vitro.

Chief Investigator: Professor David Ricketts

Principal Investigator: Professor David Ricketts

Tayside Ref: 2011DE03 **NRS Ref: N/A**

REC Ref: 11/AL/0317

EudraCT Ref: N/A **CTA Ref: N/A**

Sponsor: University of Dundee and NHS Tayside

Funder: Unfunded

Many thanks for your application to carry out the above project here in NHS Tayside. I am pleased to confirm that the project documentation (as outlined below) has been reviewed, registered and Management Approval has been granted for the study to proceed locally in Tayside.

Approval is granted on the following conditions:-

- ALL Research must be carried out in compliance with the Research Governance Framework for Health & Community Care, Health & Safety Regulations, data protection principles, statutory legislation and in accordance with Good Clinical Practice (GCP).
- All amendments to be notified to TASC R & D Office.
- All local researchers must hold either a Substantive Contract, Honorary Research Contract, Honorary Clinical Contract or Letter of Access with NHS Tayside where required (http://www.nihr.ac.uk/systems/Pages/systems_research_passports.aspx).
- TASC R & D Office to be informed of change in Principal Investigator, Chief Investigator or any additional research personnel locally.

Version 2 – 26/11/10

- Notification to TASC R & D Office of any change in funding.
- As custodian of the information collated during this research project you are responsible for ensuring the security of all personal information collected in line with NHS Scotland IT Security Policies, until destruction of this data.
- Recruitment numbers on a quarterly basis to be reported to TASC R & D Office.
- Annual reports are required to be submitted to TASC R & D Office with the first report due 12 months from date of issue of this management approval letter and at yearly intervals until completion of the study.
- Notification of early termination within 15 days or End of Trial within 90 days followed by End of Trial Report within 1 year to TASC R & D Office.
- You may be required to assist with and provide information in regard to audit and monitoring of study.

Please note you are required to adhere to the conditions, if not, NHS management approval may be withdrawn for the study.

Approved Documents

Document	Version	Date
Protocol	**1**	**09/05/11**
IRAS R & D Form (80921/213659/14/237)		**13/05/11**
IRAS SSI Form (80921/213664/5/275/108320/212932)		**13/05/11**
Letter from Sponsor		**13/04/11**
Investigator CV		**12/05/11**
CV – Dr Tamer Saleh AlJamaan		**12/05/11**
Evidence of Insurance		**13/04/11**

May I take this opportunity to wish you every success with your project.

Please do not hesitate to contact TASC R & D Office should you require further assistance.

Yours sincerely,

Elizabeth Coote
R&D Manager

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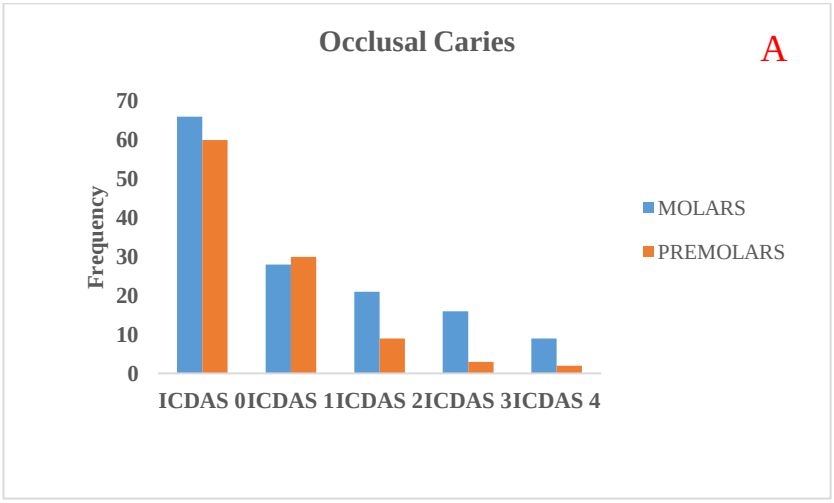
c.c. Dr Tamer Saleh AlJamaan
Dr Catrina Forde
Mrs F Bain

Version 2 – 26/11/10

Appendix 2.2

Consensus scores were those when two or more examiners agreed. In cases where all three examiners disagreed, consensus was made by discussion. Figure 2.1 and 2.2 shows the distribution of the preliminary visual and radiographic scores determined by three examiners of the occlusal and proximal surfaces of the teeth selected (from approximately 400 teeth) for use in this study.



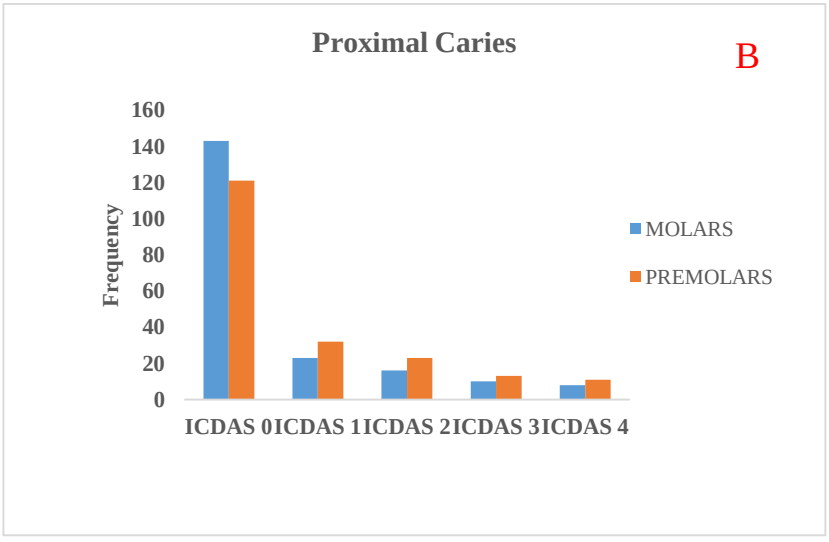
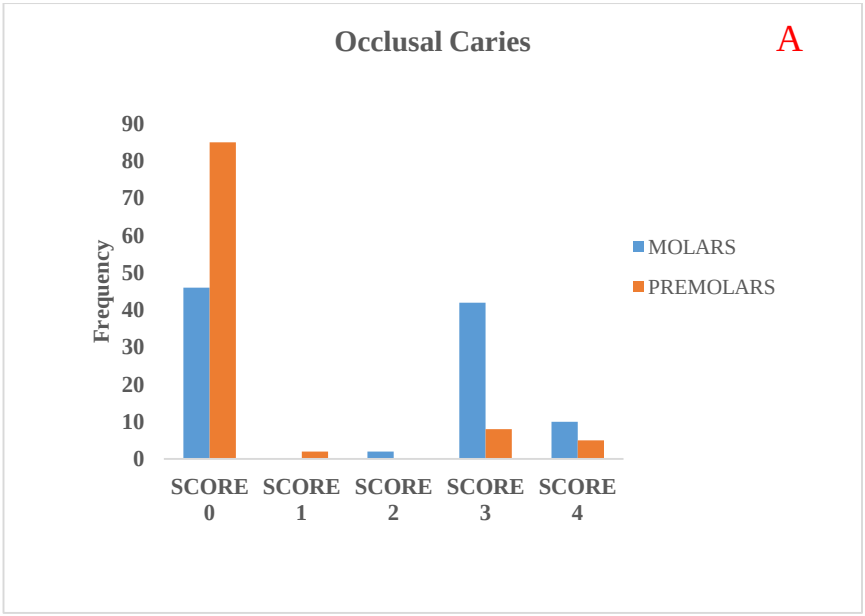


Figure 2.1 Frequency distribution of final consensus ICDAS scores of n= 244 investigation sites on occlusal surfaces (A) and n= 400 proximal surfaces (B) on molar and premolar teeth.



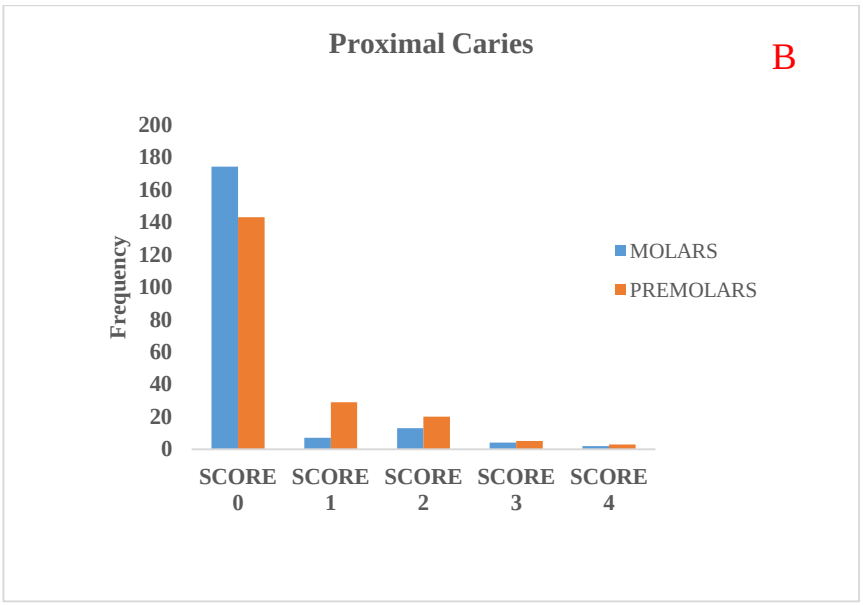


Figure 2.2 Frequency distribution of final consensus radiographic scores of n= 200 occlusal surfaces (A) and n= 400 proximal surfaces (B) on molars and premolar teeth.

Appendix 4.1

			Histology Occlusal N= 140 (172 sites)											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	71	73	86	81	70	70	82	78	71	85	82	**78** (6.3)
		D3	71	61	84	63	67	67	72	66	72	71	72	**70** (6.1)
		D3*	51	33	62	38	56	54	42	33	32	42	34	**43** (10)
	Spec. %	**D1**	86	79	74	74	79	81	72	82	73	76	74	**77** (4.5)
		D3	84	86	68	84	83	82	89	84	82	82	84	**83** (5.2)
		D3*	96	93	90	94	93	93	88	90	88	90	86	**91** (3)
FOTI	Sens. %	**D1**	72	70	66	65	75	55	71	70	56	52	58	**65** (8)
		D3	18	26	18	32	36	32	22	16	26	28	22	**25** (6.5)
	Spec. %	**D1**	82	88	79	86	79	100	79	76	76	75	80	**82** (7.3)
		D3	90	88	90	84	84	86	90	96	90	88	88	**89** (3)
BW	Sens. %	**D1**	22	18	30	12	11	15	26	31	25	12	31	**21** (7.9)
		D3	36	30	41	17	12	36	29	31	30	21	33	**29** (8.7)
	Spec. %	**D1**	87	87	83	96	96	89	96	82	86	92	86	**89** (5.2)
		D3	86	86	84	97	98	86	84	86	84	92	86	**88** (5.1)
CBCT	Sens. %	**D1**	44	71	76	46	60	69	62	58	70	48	62	**61**(10.8)
		D3	84	91	96	82	82	86	82	86	78	82	78	**84** (5.4)
	Spec. %	**D1**	96	77	66	93	90	87	76	86	82	94	77	**84** (9.3)
		D3	90	72	66	75	97	88	68	76	86	90	82	**81**(10.1)
Panoramic CBCT	Sens. %	**D1**	19	33	25	13	23	25	22	19	16	21	26	**22** (5.4)
		D3	43	46	25	19	33	32	29	40	42	33	21	**33** (9.1)
	Spec. %	**D1**	93	73	90	100	88	90	89	92	92	88	86	**89** (6.5)
		D3	97	76	88	98	92	88	88	90	86	80	92	**89** (6.5)
DD	Sens. %	**D1**	29	28	30	33	-	-	-	-	-	-	-	**33** (2.2)
		D3	21	27	21	25	-	-	-	-	-	-	-	**24** (3)
	Spec. %	**D1**	100	100	100	98	-	-	-	-	-	-	-	**100** (1)
		D3	98	98	98	98	-	-	-	-	-	-	-	**98** (0)
CarieScan PRO	Sens. %	**D1**	82	85	87	86	80	89	76	71	82	78	88	**82** (5.6)
		D3	34	38	27	17	24	29	28	24	22	16	30	**26** (6.6)
	Spec. %	**D1**	27	23	26	38	26	22	32	28	24	22	18	**26** (5.4)
		D3	93	88	93	92	98	98	92	96	92	98	90	**94** (3.4)

Table 4.1 The sensitivity and specificity for eleven examiners and seven diagnostic techniques at the D_1 and D_3 diagnostic threshold for occlusal caries diagnosis as determined by histology for 140 teeth (172 investigation sites). D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.2

			Micro-CT Occlusal N= 140 (172 sites)											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	78	73	88	82	74	76	79	88	76	86	80	**80** (5.4)
		D3	72	64	86	62	65	66	76	67	70	76	74	**71** (7)
		D3*	52	36	64	36	52	56	46	36	36	46	38	**45** (9.8)
	Spec. %	**D1**	84	78	72	76	77	82	78	78	82	74	74	**78** (3.7)
		D3	86	88	72	76	82	83	78	75	78	81	82	**80** (4.8)
		D3*	82	89	94	88	90	90	88	90	90	88	86	**89** (2.7)
FOTI	Sens. %	**D1**	75	71	75	69	79	63	72	69	62	61	66	**69** (5.8)
		D3	20	26	22	32	32	28	24	22	28	28	24	**26** (4)
	Spec. %	**D1**	80	86	82	78	86	94	86	78	78	72	86	**82** (6)
		D3	86	84	88	86	86	88	88	92	98	86	86	**88** (3.9)
BW	Sens. %	**D1**	27	22	33	14	12	16	27	32	27	16	33	**24** (7.9)
		D3	37	31	41	17	14	37	31	32	31	33	34	**31** (8.2)
	Spec. %	**D1**	94	90	84	96	100	95	96	86	86	94	90	**92** (5.1)
		D3	89	90	85	96	97	88	86	86	86	89	88	**89** (4)
CBCT	Sens. %	**D1**	53	72	77	56	74	68	63	58	72	49	66	**64** (9.3)
		D3	85	89	91	83	88	89	80	88	80	79	76	**84** (5)
	Spec. %	**D1**	96	79	69	94	92	83	77	86	80	96	76	**84** (9.1)
		D3	91	72	64	74	98	90	70	77	90	88	84	**82**(10)
Panoramic CBCT	Sens. %	**D1**	23	36	28	15	24	26	22	19	18	24	29	**24** (5.8)
		D3	41	41	32	21	28	34	31	30	38	44	23	**32** (7.5)
	Spec. %	**D1**	94	75	90	98	84	86	90	90	90	80	82	**87** (6.6)
		D3	96	74	89	98	92	88	90	90	90	78	89	**89** (7)
DD	Sens. %	**D1**	31	33	33	33	-	-	-	-	-	-	-	**33** (1.3)
		D3	22	22	25	27	-	-	-	-	-	-	-	**24** (2.4)
	Spec. %	**D1**	95	95	94	89	-	-	-	-	-	-	-	**93** (2.9)
		D3	97	97	97	96	-	-	-	-	-	-	-	**97** (0.5)
CarieScan PRO	Sens. %	**D1**	78	89	82	86	84	88	72	74	80	76	82	**81** (5.6)
		D3	38	27	26	16	23	27	30	26	24	18	31	**26** (6)
	Spec. %	**D1**	32	18	28	32	22	18	36	22	28	22	16	**25** (6.7)
		D3	95	88	93	98	98	98	96	94	96	92	94	**95** (3)

Table 4.2 The sensitivity and specificity for eleven examiners and seven diagnostic techniques at the D_1 and D_3 diagnostic threshold for occlusal caries diagnosis as determined by Micro-CT for 140 teeth (172 investigation sites). D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.3

			Histology Occlusal N= 140											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	80	76	90	82	74	78	84	76	82	84	84	**81** (4.7)
		D3	81	64	82	66	69	68	76	68	71	70	76	**72** (6)
		D3*	52	36	64	33	58	52	48	36	34	46	38	**45** (10)
	Spec. %	**D1**	84	78	70	70	82	80	70	80	72	74	74	**76** (5.2)
		D3	82	86	70	82	84	80	88	82	80	82	82	**82** (4.5)
		D3*	90	91	98	89	95	93	90	92	88	90	88	**91** (3.1)
FOTI	Sens. %	**D1**	78	74	70	66	74	60	70	72	58	50	66	**67** (8.3)
		D3	20	30	22	30	38	32	26	18	24	32	28	**27** (6)
	Spec. %	**D1**	80	86	82	84	82	100	78	78	74	76	82	**82** (6.9)
		D3	90	90	89	85	82	88	90	96	89	86	88	**88** (3.5)
DD	Sens. %	**D1**	27	29	31	32	-	-	-	-	-	-	-	**31** (2.2)
		D3	22	29	22	24	-	-	-	-	-	-	-	**24** (3.3)
	Spec. %	**D1**	100	100	100	98	-	-	-	-	-	-	-	**100** (1)
		D3	98	98	98	99	-	-	-	-	-	-	-	**98** (0.3)
CarieScan PRO	Sens. %	**D1**	80	84	86	88	82	88	80	72	84	80	88	**83** (4.8)
		D3	32	36	29	18	22	32	26	26	20	18	30	**26** (6.1)
	Spec. %	**D1**	28	24	26	36	28	24	32	26	28	26	20	**27** (4.2)
		D3	90	90	90	94	98	98	90	98	90	98	92	**93** (3.8)

Table 4.3 The sensitivity and specificity for eleven examiners and four diagnostic techniques at the D_1 and D_3 diagnostic threshold for occlusal caries diagnosis as determined by histology for 140 teeth. D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.4

			Micro-CT Occlusal N= 140											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	80	76	86	84	76	72	80	90	84	88	82	**82** (5.5)
		D3	76	68	82	68	72	70	78	72	74	76	78	**74** (4.3)
		D3*	54	38	62	42	48	58	52	36	38	48	32	**46** (9.7)
	Spec. %	**D1**	86	80	70	74	78	80	80	76	80	78	74	**78** (4.2)
		D3	88	90	70	72	80	86	80	72	76	80	83	**80** (6.7)
		D3*	88	90	94	88	90	90	86	90	90	84	86	**89** (2.5)
FOTI	Sens. %	**D1**	78	72	73	66	80	68	76	71	68	64	70	**71** (5)
		D3	22	28	26	30	34	30	22	26	30	30	24	**28** (3.8)
	Spec. %	**D1**	80	82	84	80	86	92	88	78	76	70	86	**82** (6.1)
		D3	88	82	88	86	86	90	88	90	98	88	86	**88** (3.7)
DD	Sens. %	**D1**	30	36	32	32	-	-	-	-	-	-	-	**33** (2.5)
		D3	28	26	24	26	-	-	-	-	-	-	-	**26** (1.6)
	Spec. %	**D1**	95	95	94	89	-	-	-	-	-	-	-	**93** (2.9)
		D3	97	97	97	96	-	-	-	-	-	-	-	**97** (0.5)
CarieScan PRO	Sens. %	**D1**	80	89	84	84	86	88	76	72	82	74	82	**82** (5.6)
		D3	42	29	28	18	20	26	26	28	22	20	28	**26** (6.5)
	Spec. %	**D1**	36	16	26	34	24	20	34	24	32	18	16	**25** (7.5)
		D3	96	88	93	88	88	98	96	94	96	92	94	**93** (3.6)

Table 4.4 The sensitivity and specificity for eleven examiners and four diagnostic techniques at the D1 and D_3 diagnostic threshold for occlusal caries diagnosis as determined by Micro-CT for 140 teeth. D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.5

			Histology Proximal N= 140 (280 surfaces)											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	50	48	51	73	57	60	73	50	48	48	51	**55** (9.5)
		D3	52	48	56	70	81	63	54	66	78	72	70	**65** (10)
		D3*	24	36	42	36	28	36	48	42	48	50	42	**39** (8.3)
	Spec. %	**D1**	95	94	90	90	92	90	88	90	90	84	90	**90** (2.9)
		D3	91	93	91	85	87	91	90	90	84	88	86	**89** (2.9)
		D3*	94	94	94	96	92	90	89	98	96	90	98	**94** (3.2)
FOTI	Sens. %	**D1**	33	34	50	30	52	45	49	52	33	48	46	**43** (8.6)
		D3	22	19	19	26	19	19	22	20	19	19	20	**20** (2.2)
	Spec. %	**D1**	99	99	98	99	99	98	99	98	97	99	98	**98** (0.7)
		D3	99	99	98	99	98	99	99	99	98	99	99	**99** (0.5)
BW	Sens. %	**D1**	27	27	37	29	18	27	19	28	32	30	32	**28** (5.5)
		D3	14	18	10	8	8	6	12	10	8	14	16	**11** (3.8)
	Spec. %	**D1**	80	68	69	78	84	74	82	78	72	74	72	**76** (5.2)
		D3	84	92	89	92	90	94	86	88	90	86	82	**88** (3.7)
CBCT	Sens. %	**D1**	44	46	57	30	46	42	44	48	52	54	48	**46** (7.1)
		D3	67	74	74	48	63	56	72	66	54	48	56	**62** (9.8)
	Spec. %	**D1**	98	89	82	98	97	97	96	98	86	88	92	**93** (5.7)
		D3	98	97	83	98	99	98	86	98	96	98	96	**95**(5.4)
Panoramic CBCT	Sens. %	**D1**	18	32	37	23	22	20	18	32	36	24	22	**26** (7.1)
		D3	26	48	55	30	30	26	40	42	46	30	40	**38** (9.8)
	Spec. %	**D1**	91	83	70	88	92	94	96	84	88	92	88	**88** (7.1)
		D3	95	88	73	92	92	90	84	90	92	92	84	**88** (6.1)
DD	Sens. %	**D1**	10	10	10	-	-	-	-	-	-	-	-	**10** (0)
		D3	13	10	10	-	-	-	-	-	-	-	-	**11** (1.7)
	Spec. %	**D1**	100	100	100	-	-	-	-	-	-	-	-	**100** (0)
		D3	96	98	97	-	-	-	-	-	-	-	-	**97** (1)

Table 4.5 The sensitivity and specificity for eleven examiners and seven diagnostic techniques at the D_1 and D_3 diagnostic threshold for proximal caries diagnosis as determined by histology for 140 teeth (280 surfaces). D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.6

			Micro-CT Proximal N= 140 (280 surfaces)											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	52	49	48	74	58	59	72	53	49	46	50	**55** (9.6)
		D3	55	52	55	72	79	66	55	68	76	72	74	**66** (9.8)
		D3*	26	40	46	38	30	44	40	46	46	56	44	**41** (8.2)
	Spec. %	**D1**	95	95	90	91	92	93	90	91	88	88	90	**91** (2.4)
		D3	91	94	92	86	87	92	93	90	91	88	88	**89** (2.6)
		D3*	90	92	98	98	96	90	89	88	96	96	94	**93** (3.7)
FOTI	Sens. %	**D1**	31	32	48	29	49	45	47	48	32	49	46	**41** (8.4)
		D3	24	17	21	20	20	22	22	20	22	24	24	**21** (2.2)
	Spec. %	**D1**	97	98	87	96	91	97	88	88	94	92	90	**93** (4.1)
		D3	100	99	98	100	98	98	98	98	100	100	98	**99** (1)
BW	Sens. %	**D1**	26	29	34	26	19	26	20	29	30	32	28	**27** (4.6)
		D3	12	18	12	10	10	9	14	8	8	16	18	**12** (3.7)
	Spec. %	**D1**	80	69	68	78	82	78	80	76	76	74	78	**76** (4.4)
		D3	89	90	92	94	90	90	90	90	92	86	84	**90** (2.8)
CBCT	Sens. %	**D1**	44	47	56	31	48	44	42	54	54	52	50	**47** (7.1)
		D3	69	77	77	48	66	55	74	66	56	52	58	**63** (10)
	Spec. %	**D1**	98	90	82	98	99	98	96	96	96	88	92	**94** (5.3)
		D3	99	98	92	100	100	98	88	94	98	98	98	**97** (3.7)
Panoramic CBCT	Sens. %	**D1**	18	33	37	26	22	22	18	34	33	26	24	**27** (6.7)
		D3	28	45	55	28	30	28	44	50	28	26	42	**37** (10)
	Spec. %	**D1**	91	84	70	89	90	92	96	86	88	90	88	**88** (6.6)
		D3	95	87	73	92	92	96	86	90	94	90	84	**89** (6.5)
DD	Sens. %	**D1**	10	12	12	-	-	-	-	-	-	-	-	**11** (1.2)
		D3	12	10	10	-	-	-	-	-	-	-	-	**12** (0)
	Spec. %	**D1**	100	98	98	-	-	-	-	-	-	-	-	**99** (1.2)
		D3	96	98	98	-	-	-	-	-	-	-	-	**97** (1.2)

Table 4.6 The sensitivity and specificity for eleven examiners and seven diagnostic techniques at the D_1 and D_3 diagnostic threshold for proximal caries diagnosis as determined by Micro-CT for 140 teeth (280 surfaces). D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.7

			Histology Occlusal N= 200 (244 sites)											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	61	71	80	75	72	70	84	76	72	78	84	**75** (6.7)
		D3	70	64	87	66	65	70	70	72	68	70	74	**71** (6.2)
		D3*	48	30	59	41	54	54	38	30	36	40	30	**42** (10)
	Spec. %	**D1**	85	78	72	75	76	78	80	78	76	78	78	**78** (3.2)
		D3	90	86	70	83	85	84	88	86	84	80	84	**84** (5.2)
		D3*	96	92	92	96	91	90	94	96	90	88	92	**92** (2.7)
FOTI	Sens. %	**D1**	63	64	87	66	65	70	70	72	68	70	74	**70** (6.6)
		D3	31	31	42	40	42	36	32	29	41	42	29	**36** (5.6)
	Spec. %	**D1**	89	87	82	90	84	90	82	78	76	74	82	**83** (5.6)
		D3	96	97	96	97	92	96	96	89	92	92	90	**94** (2.9)
BW	Sens. %	**D1**	17	17	29	8	10	12	22	28	19	14	26	**18** (7.2)
		D3	33	27	39	14	10	33	32	28	28	24	28	**27** (8.4)
	Spec. %	**D1**	89	93	84	96	96	92	90	92	88	90	92	**91** (3.5)
		D3	91	91	85	98	98	90	88	96	84	94	90	**91** (4.8)
CBCT	Sens. %	**D1**	40	63	65	40	62	66	64	52	66	52	58	**57** (9.8)
		D3	84	92	94	76	78	82	86	82	82	78	72	**82** (6.6)
	Spec. %	**D1**	93	79	66	93	92	88	70	72	90	92	90	**84** (10)
		D3	90	78	70	76	96	88	70	88	90	92	90	**84**(9.2)
Panoramic CBCT	Sens. %	**D1**	16	26	22	11	26	22	20	16	14	21	24	**20** (5)
		D3	41	41	28	24	36	34	28	42	40	30	32	**34** (6.3)
	Spec. %	**D1**	93	74	95	98	86	80	88	94	94	90	86	**89** (7.1)
		D3	97	85	86	87	90	86	88	88	84	82	86	**87** (3.9)
DD	Sens. %	**D1**	29	27	28	31	-	-	-	-	-	-	-	**29** (1.7)
		D3	20	26	23	23	-	-	-	-	-	-	-	**23** (2.4)
	Spec. %	**D1**	96	99	96	96	-	-	-	-	-	-	-	**97** (1.5)
		D3	96	98	98	97	-	-	-	-	-	-	-	**97** (1)
CarieScan PRO	Sens. %	**D1**	79	84	87	85	82	88	72	72	80	76	86	**81** (5.7)
		D3	37	43	31	18	22	26	22	26	20	12	28	**26** (8.8)
	Spec. %	**D1**	21	18	24	28	20	20	26	26	20	22	20	**22** (3.2)
		D3	94	86	93	93	97	96	92	94	90	97	90	**93** (3.3)

Table 4.7 The sensitivity and specificity for eleven examiners and seven diagnostic techniques at the D1 and D3 diagnostic threshold for occlusal caries diagnosis as determined by histology for 200 teeth (244 investigation sites). D3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.8

			Histology Occlusal N= 200											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	63	74	86	70	70	72	82	74	82	82	82	76 (7.1)
		D3	71	66	82	68	72	72	78	68	72	70	66	71 (4.9)
		D3*	46	28	58	36	56	52	38	32	34	46	36	42 (10)
	Spec. %	**D1**	82	80	74	72	86	82	72	76	74	72	76	77 (4.8)
		D3	84	88	72	86	84	80	90	90	86	82	82	84 (4.1)
		D3*	92	94	98	90	92	90	92	92	90	90	86	91 (3)
FOTI	Sens. %	**D1**	60	64	76	66	68	68	70	72	62	66	68	67 (4.5)
		D3	29	28	36	36	38	32	36	20	44	40	28	33 (6.8)
	Spec. %	**D1**	90	86	84	94	82	98	78	78	76	70	84	84 (8.2)
		D3	96	98	94	92	92	98	92	92	92	88	88	93 (3.4)
DD	Sens. %	**D1**	27	27	30	31	-	-	-	-	-	-	-	29 (2.1)
		D3	18	24	22	24	-	-	-	-	-	-	-	22 (2.8)
	Spec. %	**D1**	96	97	96	95	-	-	-	-	-	-	-	96 (0.8)
		D3	97	97	96	95	-	-	-	-	-	-	-	96 (1)
CarieScan PRO	Sens. %	**D1**	80	84	88	88	82	86	80	72	82	82	86	83 (4.6)
		D3	30	34	30	16	22	30	28	22	24	18	32	26 (5.9)
	Spec. %	**D1**	20	18	22	26	20	18	28	26	20	18	22	22 (3.6)
		D3	92	90	90	88	92	96	90	92	88	98	86	91 (3.5)

Table 4.8 The sensitivity and specificity for eleven examiners and four diagnostic techniques at the D_1 and D_3 diagnostic threshold for occlusal caries diagnosis as determined by histology for 200 teeth. D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 4.9

			Histology Proximal N= 200 (400 surfaces)											
			EX 1	EX 2	EX 3	EX 4	EX 5	EX 6	EX 7	EX 8	EX 9	EX 10	EX 11	Mean (SD)
ICDAS	Sens. %	**D1**	48	48	49	66	54	58	70	60	46	46	49	**54** (8.4)
		D3	52	48	58	70	78	62	56	68	72	72	68	**64** (9.5)
		D3*	22	30	41	33	26	36	42	46	46	46	38	**37** (8.3)
	Spec. %	**D1**	96	96	93	94	92	93	90	94	92	86	92	**93** (2.8)
		D3	94	95	94	89	89	92	92	92	86	92	92	**92** (2.6)
		D3*	99	98	95	98	92	92	96	92	94	90	96	**95** (3)
FOTI	Sens. %	**D1**	32	33	51	30	53	46	47	54	36	46	48	**43** (8.8)
		D3	22	19	19	26	19	18	24	22	19	19	22	**21** (2.6)
	Spec. %	**D1**	97	98	93	97	94	94	98	89	90	92	92	**94** (3.2)
		D3	99	99	98	99	99	99	98	99	99	98	99	**99** (0.3)
BW	Sens. %	**D1**	24	23	35	26	15	24	20	26	28	26	30	**25** (5.2)
		D3	15	15	8	5	5	6	10	8	8	12	12	**9** (3.9)
	Spec. %	**D1**	81	72	69	81	82	70	86	72	68	70	76	**75** (6.3)
		D3	94	92	82	97	92	88	88	90	86	82	86	**89** (4.8)
CBCT	Sens. %	**D1**	41	44	54	29	44	44	40	44	46	50	46	**44** (6.3)
		D3	67	74	74	48	62	58	74	68	56	56	58	**63** (8.8)
	Spec. %	**D1**	97	93	82	97	92	88	88	90	86	82	86	**89** (5.2)
		D3	98	97	91	99	99	98	92	98	98	98	96	**97** (2.7)
Panoramic CBCT	Sens. %	**D1**	17	32	36	22	21	18	16	34	32	22	19	**24** (7.5)
		D3	26	48	56	30	32	24	32	40	42	30	39	**36** (9.8)
	Spec. %	**D1**	92	88	71	87	94	96	92	86	92	92	90	**89** (6.7)
		D3	96	90	74	91	92	90	86	90	92	92	88	**89** (5.6)
DD	Sens. %	**D1**	13	12	8	-	-	-	-	-	-	-	-	**11** (2.6)
		D3	13	8	8	-	-	-	-	-	-	-	-	**10** (2.9)
	Spec. %	**D1**	93	98	96	-	-	-	-	-	-	-	-	**96** (2.5)
		D3	99	97	99	-	-	-	-	-	-	-	-	**98** (1.2)

Table 4.9 The sensitivity and specificity for eleven examiners and seven diagnostic techniques at the D_1 and D_3 diagnostic threshold for proximal caries diagnosis as determined by histology for 200 teeth (400 surfaces). D_3* denotes ICDAS at cut-off point between 2 and 3.

Appendix 5.1

Volumetric assessment of occlusal caries lesions using Micro-CT: A methodological pilot study

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Micro-CT is a 3D non-destructive imaging system which can be used to visualise and quantitatively assess dental hard tissues and carious lesions. Most studies have analysed individual tomographic cuts. This study aimed to devise a quantitative method for volumetric analysis of occlusal lesions from Micro-CT images and to determine the relationship between ICDAS scores and lesion volume.

Twenty extracted teeth were selected with varying appearance from sound to non-cavitated carious lesions on the occlusal surface. Three trained examiners independently examined one investigation site per tooth according to the ICDAS criteria (www.ICDAS.org); consensus scores were used in analyses. Teeth were scanned using a desktop µCT 40 Scanco system with an isotropic resolution of 20 µm. One examiner measured the total volume of the occlusal lesion in enamel and dentine (when present) at each investigation site using a Photoshop CS6 software programme and a modified script to measure the sum of pixels after slicing the tooth in 0.1 mm thickness slices and setting the threshold to the lowest Linear attenuation coefficient (LAC) of enamel and dentine.

The mean volume of demineralised **enamel** in the lesions detected according to ICDAS scores were 0.131 mm^3 for code 1 (n= 8), 0.334mm^3 for code 2 (n=4) and 1.88 mm^3 for

score 3 (n=3). The mean total volume of demineralised **enamel and dentine** in the lesions detected according to ICDAS codes 1, 2 and 3 were 0.131 mm^3, 0.525mm^3 and 9.63 respectively. One way ANOVA showed that there were significant difference in lesion volume between ICDAS scores (P value < 0.05).

In conclusion, a method of volumetric analysis of carious lesions using micro-CT has been devised which shows a difference in lesion volume between ICDAS scores.

Appendix 5.2

Photoshop Script for Volume of Caries Measurement

```
#target photoshop
//$.level = 2;
//debugger; // launch debugger on next line
app.displayDialogs = DialogModes.NO;
var startRulerUnits = app.preferences.rulerUnits;
app.preferences.rulerUnits = Units.PIXELS;
var docRef = app.activeDocument;
//var fileOut = new File
("~/Desktop/TamerOutput/histogramTest01.txt");
var fileOut =  File.saveDialog("Please save as text, later
open in excel");
fileOut.lineFeed = "Windows";
fileOut.open("w","TEXT","????");
fileOut.write("histogram process...." + docRef.name +
"\n");
var myChannels = docRef.channels;
//rename channels
For (var channelIndex = 0; channelIndex <
myChannels.length; channelIndex++)
{myChannels[channelIndex].name = "slice00" + channelIndex;}
// find out how many pixels I have
var totalCount = docRef.width.value * docRef.height.value;
fileOut.write("Total pixel count is: " + totalCount +
"\n");
fileOut.write("\n\n\n\n");
//turn off all channels first
For (var channelIndex = 0; channelIndex <
myChannels.length; channelIndex++) {var currentChannel =
myChannels[channelIndex]; currentChannel.visible = false;}
```

```
var volumePixelTotal = 0;
For (var channelIndex = 0; channelIndex <
myChannels.length; channelIndex++)
{//myChannels[channelIndex].name = "slice00" +
channelIndex;var currentChannel =
myChannels[channelIndex];currentChannel.visible = true var
currentHistogram = currentChannel.histogram;
    fileOut.write(currentChannel.name);
    var thresholdValue = 128; var blackTotal = 0; var
whiteTotal = 0;
   //output the histogram
    For (var histogramInx = 0; histogramInx < current
Histogram.length; histogramInx++) {if (histogram Inx <
threshold Value   blackTotal += current
Histogram[histogramInx];}else{whiteTotal+=currentHistogram[
histogramInx];}
          //end for each level in histogram
   fileOut.write("," + blackTotal);
   fileOut.write("," + whiteTotal);
   volume Pixel Total += whiteTotal; //sum this white pixel
column
   current Channel.visible = false;
   fileOut.write("\n");
   //end for each channel
  fileOut.write("\n\n Total number of white pixels in
Volume is:,," +      volumePixelTotal );
fileOut.write("\n\n Total volume in millimeter is:,," +
volumePixelTotal * 0.02 *0.02 *0.1 );
fileOut.close(); app.preferences.rulerUnits =
startRulerUnits;
```